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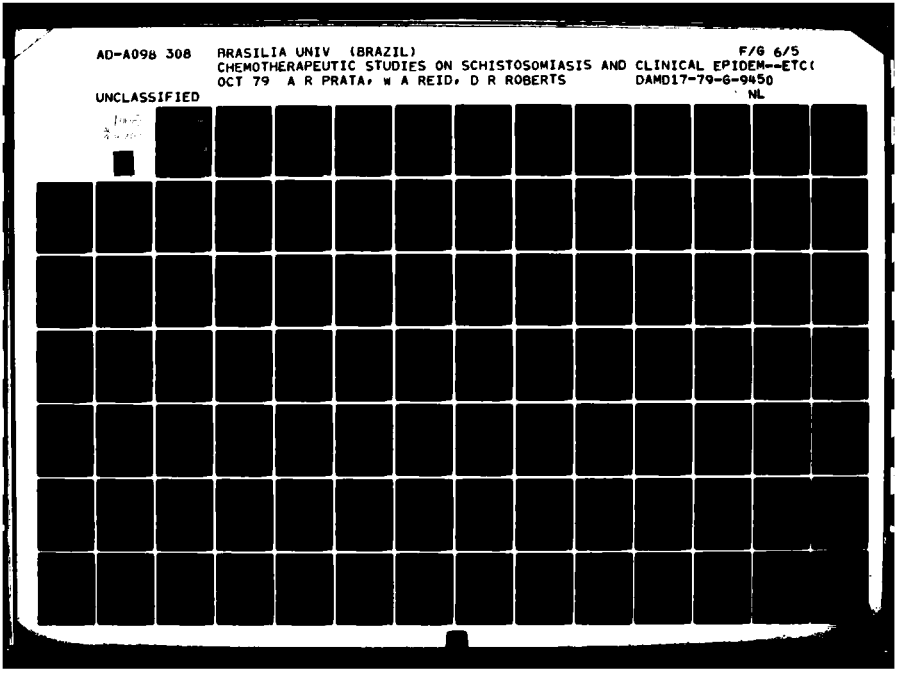

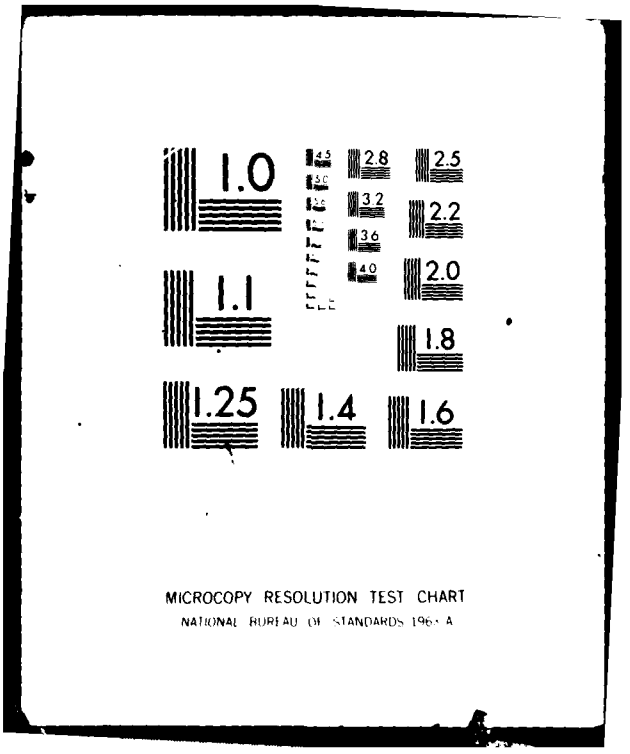
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CHEMOTHERAPEUTIC STUDIES ON SCHISTOSOMIASIS AND CLINICAL EPEDEMIOLOGICAL AND IMMUNOLOGICAL STUDIES ON MALARIA IN AMAZONAS, BRAZIL, ALONG THE ITUXI RIVER

FINAL REPORT

(1) LEVEL II

Aluizio R. Prata
Willis A. Reid, JR.
Donald R. Roberts
K. Mills McNeill

October 1979

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US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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Universidade de Brasilia
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Chemotherapy	Entomology

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Objectives are to find new prophylactic and curative drugs for the prevention and cure of schistosomiasis infections and to study the clinical, epidemiologic, drug susceptibility and vector transmission patterns of falciparum malaria in the Amazon River basin of Brazil. Both are primary diseases which would be acquired by US Military and DOD personnel in the event of deployment to any of numerous tropical areas.

Prophylactic(PMT) and curative (PCT) testing (in mice) against schistosomiasis

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for candidate compounds submitted by the WRAIR Anti-Schistosomal Drug Testing Program. Compounds active in the primary screen are extensively reexamined for confirmation and dose response patterns. The malaria immunology studies include the testing of sera from endemic areas by the indirect fluorescent antibody test, in vitro drug susceptibility testing and creation of a cryobank of human strains of Plasmodium falciparum. Malaria vector transmission studies include field and laboratory analysis of morphological, behavioral, physiological and DDT susceptibility patterns of Anopheles darlingi and other potential anophelene malaria vectors.

During the reporting period 1065 compounds were screened in the PCT and PMT. Of these 58 were designated as confirmed or unconfirmed active and 232 were designated toxic. A malaria serological laboratory is now fully operational and providing logistical support for ongoing malaria field studies. Field collection, cryopreservation and in vitro cultivation and drug susceptibility testing of Plasmodium falciparum was accomplished. Complete entomological surveys were conducted at the field study sites. An. darlingi was found to be susceptible to DDT; movement, host seeking, biting activity and endophagic behavior was characterized.

B

PART I. CHEMOTHERAPEUTIC STUDIES ON SCHISTOSOMIASIS.

1. Description: Schistosomiasis continues to be ranked as one of the most important of the tropical diseases, yet we still lack suitable means for chemotherapeutic management. The few drugs currently available demonstrate only partial curative efficacy and are often accompanied by adverse side effects ranging from carcinogenicity to headaches and dizziness. Considering the actual and potential global commitments of United States military and civilian personnel, the risks to infection with one of the human schistosomes remains high. Indeed the incidence of infection within foci of local indigenous populations may approach 100 percent. Consequently, a major research effort in anti-schistosomal drug development is being carried out by the Walter Reed Army Institute of Research (WRAIR) in conjunction with the University of Brasilia (USAMRU-Brasilia). The test compounds are obtained from the Division of Experimental Therapeutics (WRAIR) and are tested for prophylactic and/or curative activity in mice at preestablished dosage levels. The ultimate objective is the identification of compounds with a high potential for use in the prevention and treatment of schistosomiasis mansoni.

2. Progress:

a. Laboratory Facilities. At the beginning of the reporting period, the WRAIR program in Belém, Brazil (USAMRU-Belém) began the phase out of its operations. As a result of this closing, the USAMRU-Brasilia program acquired by transfer numerous items of equipment and laboratory and field supplies that have greatly enhanced the on-going schistosomiasis and malaria research efforts. Such key major items as microscopes, autoclave, vacuum pumps and centrifuge have been especially beneficial to the schistosomiasis program. Many expendable laboratory supplies were also acquired which significantly delayed the necessity to order for restocking current levels. Another major improvement in laboratory operations was the installation of a central air compressor in the Nucleo de

Medicina Tropical e Nutrição, thus providing a reliable source of aeration to the snail colony. Plans have also been submitted and approved for the construction of a small isolation room in the Pharmacy. A chemical exhaust hood will be installed in this area. These improvements will provide a safer area for the preparation of test drugs for anti-schistosomal therapy. We have also prepared a request for the purchase of an ultrasonic disintegrator system to be used in mixing compounds to assure an adequate drug suspension mixture in the designated vehicle. This will considerably improve the accuracy of drug dosage administration.

b. Animal Facilities. In FY78 we reported serious problems with the weekly supply of healthy mice from the University of Brasilia Bioterio (vivarium). The Bioterio has problems with rearing young mice to an acceptable weight (18-23 grams) and many of these are ill with a broad spectrum of parasitic, bacterial and/or viral infections. More recently, for example, there was an unusually high mortality of young mice from what appears to be ectromelia (mouse pox). In March and April of this year LTC Robert J. Beattie VC, Chief of the Department of Animal Resources, WRAIR, visited USAMRU-Brasilia at the request of the University of Brasilia. He engaged in extensive consultations with Dr. Andre de Mello, Chief of the UnB Bioterio, and they jointly published a report covering analyses of the problems, and recommended actions to bring the quality of animal management and facilities to acceptable standards. Those recommendations are now being studied by University officials and we hope soon to begin extensive physical renovations to the current facility. Such renovation should include at a minimun: 1) complete isolation of animal rooms from the outside environment to include separation of "clean" areas from "dirty" areas; 2) wash area with the provision of hot water and sterilization facilities; 3) control of air circulation, temperature and relative humidity in animal rooms; 4) adequate storage areas for food and bedding; and 5) reorganization of the personnel management structure for Bioterio animal technical personnel.

Current animal facilities within the drug testing laboratory are adequate for maintaining experimental mice. We continue to use ground corn-cob (cellulose) bedding material purchased annually from a local farmer. We are presently devising a method for improving this by filtering out the fine dust portion of the finished ground product.

c. Snail Colony. The Biomphalaria glabrata (Paulista Strain) snail colony continues to provide Schistosoma mansoni cercariae in sufficient quantities to perform the weekly mouse exposures for drug testing and life cycle maintenance. A weekly average of 400 snails were exposed to miracidia recovered from macerated

infected mouse livers. An unusually low level of prepatent mortality (5 percent) was obtained; of the survivors which were screened 42 days post-exposure, 57 percent were positive for emerging cercariae. These were maintained for future cercariae collections and a weekly average population of 1,200 positive snails (range 804 to 1,853) were on hand at one time. In general approximately 58 percent of the snails exposed were later recovered with patent infections.

d. Drug Testing. A total of 690 bottle number compound samples were received from WRAIR during the reporting period. Of these 5 compounds were predesignated for both prophylactic (PMT) and curative (PCT) testing; 557 compounds were predesignated for only curative testing and 128 compounds were predesignated for only mortality testing. Table 1 summarizes the workload data relative to drug testing. While 474 specific compounds were tested in the PCT, for example, 135 of those were compounds which were actually received during FY79. All others were tests of compounds received earlier and representing a backlog. The situation with the PMT testing backlog was more drastic. No compounds received during FY79 were actually tested during the reporting period; all compounds tested in the PMT were retests or tests of compounds received earlier than 1 Oct 78. The reasons for the accumulated backlogs are twofold: 1) increased emphasis on retesting compounds which show initial activity or toxicity, and 2) reduced numbers of mice available for weekly testing (see "Animal Facilities" above). Both factors worked to accumulate a backlog while compounds continued to be shipped from WRAIR. Additionally, many promising compounds were tested under "dose response" conditions at two routes of administration (subcutaneously or orally by gavage). Consequently, one compound test might involve the use of as many as 12 groups of test mice (70 mice). Indeed, one PMT run (PMT 78339) consisting of 250 test mice evaluated only 5 compounds under these conditions. All such retest backlogs have been eliminated and we are currently diminishing the untested drug backlog.

e. Personnel. The drug testing program is directed by one American Senior Investigator and supported by a staff of seven Brazilian Laboratory Assistants and one secretary/typist. The operating program is broken down into five work areas: 1) Snail Colony (two people); 2) Animal Service (one person); 3) Necropsy (one person); Pharmacy (two people); and Administration (two people). All individuals are cross-trained in procedures of snail maintenance, subcutaneous and gavage drug administration, daily mouse maintenance with mortality checks, and mouse exposures to cercariae. Each individual is able to perform all duties in at least two other areas of work. One of the persons listed under "Administration" is a

TABLE 1

FY78 workload data summary for USAMRU-Brasilia anti-shistosome drug testing program.

Workload Criterion	PMT	PCT	Total
Number of Test Runs	24	15	39
Number of Untreated, Uninfected Control Groups*	30	48	78
Number of Untreated, Infected Control Groups*	126	60	186
Number of Reference Drug Groups*	43	45	88
Number of Test Drug Groups*	849	595	1444
Number of Drugs Tested (Total Bottle Number Compounds)	591	474	1065
Number of Drugs Inactive and Non-Toxic (See Tables and)	400	375	775
Number of Drugs Toxic (See Tables and)	173	59	232
Number of Drugs Active (Confirmed or Unconfirmed) (See Tables and)	18	40	58
Total Number of Mice Utilized	NA	NA	13830
Drug Testing	5330	3650	8980
Life Cycle	NA	NA	3400
Other	NA	NA	1400

* One group contains 5 test mice.

senior laboratory technician capable of performing duties in all laboratory work areas as the need arises.

3. Test Procedures:

a. General: The current system of testing places a priority of prophylactic or curative testing on each compound. For those compounds received for testing in both systems, prophylactic testing is still performed first. All tests, prophylactic or curative, are performed with groups of five mice per drug per dosage. All mice are individually tail-exposed for 30 minutes to the numbers of cercariae required by the specific test. Drugs are routinely prepared for administration in a peanut oil vehicle unless another vehicle (such as water, saline, alcohol, or cremophor) has been previously recommended. All drugs are administered subcutaneously unless orally (by gavage) has been previously designated. Likewise, all drugs are administered in terms of mgs per kg body weight of mouse recipient.

b. Primary Mortality Test (PMT): The PMT is a prophylactic test in that it evaluates drug activity against immature migrating larval schistosomes. Mice are exposed to 3,000 - 3,500 S. mansoni cercariae. Two days after exposure, drugs are administered in a single innoculation to each of the five test animals per drug. The standard initial test dose is 640 mgs/kg and future testing may repeat this dose, with other groups being tested at lower dosages.

For every PMT group there are control groups of 1) 25 infected untreated mice, 2) 10 normal mice, and 3) five mice treated with the reference drug Niridazole (640 mgs/kg). The infected untreated control mice will begin dying on day 20 post-exposure and none will survive past day 30 in most cases. Niridazole-treated mice survive until day 49. Active drugs are those for which treated mice survive two weeks after the mean day of death of the infected control mice. At 49 days, all surviving mice (controls and drug test) are perfused for total worm burden determination (1). Drugs are considered toxic at the dosage given if recipient mice die within 10 days post-treatment (12 days post-exposure). All active compounds are scheduled for later retest confirmation at the same dosage and route of administration as the initial test. If positive confirmation is obtained for activity, then further testing at different dosages by both routes (subcutaneous or oral) is scheduled. Toxic compounds are retested at lower dosages until non-toxicity is obtained.

c. Primary Curative Test (PCT): The PCT is a curative test of a compound against an established S. mansoni infection in mice exposed to 160-200 cercariae. Thirty-three days post-exposure drugs

(100 mgs/kg) are administered daily for five consecutive days (until day 37) in the same manner as described for the PMT. Three days following the last treatment, all mice are: 1) killed individually by cervical dislocation; 2) the livers are immediately removed; 3) the livers are made into liver squash preparations; and 4) the numbers and condition of worms in the liver are determined for each surviving animal. Control groups for each PCT run are: 1) 20 untreated infection control mice; 2) 10 Niridazole treated mice (5 at 100 mgs/kg/day and 5 at 160 mgs/kg/day) and 3) five Oxamniquine treated mice (100 mgs/kg/day).

Criteria for drug activity are based upon the hepatic shift of adult worms from the mesenteries to the liver. This shift is presumed to be a result of drug pressure. Not only are the total numbers of liver worms determined but the conditions of those worms are also taken into consideration. The presence of dead worms is incontrovertable evidence of drug activity, while the presence of small, abnormally developed "sick" worms, possessing little movement, is evidence of possible activity requiring further testing.

Untreated control mice will normally show five to 15 worms (with a mean of 11 to 13 worms) in the liver. A mean test animal liver worm burden of 20 to 25 worms may be indicative of "marginal" drug activity. Such mean burdens higher than 25, even though the worms are living, is justification for retesting, possibly at a higher dosage level. Oxamniquine treatment produces high dead worm burdens in the livers of infected animals while Niridazole produces high living (but "sick") worm burdens at 100 mgs/kg and 160 mgs/kg, with the appearance of a few dead worms at the latter dose.

d. Secondary Curative Test (SCT): We reported earlier (Annual Technical Report for FY78) our efforts to expand the drug testing capabilities to secondary curative testing. At that time we were standardizing the test and formulating the data interpretation criteria. Those standardizations have been completed and we are prepared to initiate testing early in FY80.

The SCT is designed to determine the minimal dose of each drug which produces the death of virtually all worms and to characterize for each drug treatment the time required to produce an effect upon the residual live worm burden. The following effects will represent partial or complete drug activity: 1) the presence of dead worms and/or 2) the presence of abnormally low total worm burdens and/or 3) the presence of abnormally developed worms ("stunted", "sick" or otherwise representing abnormal morphologic development). This will be confirmed by more refined microscopic analysis. Table 2 depicts the proposed standard protocol for the SCT. As can be

seen from the numbers of animals required to test one drug at one dose, careful selection of candidate compounds must be made based upon prior results in the PCT in order to take maximum advantage of the test system.

Control infections in the SCT should behave as follows:

1) Infected, untreated controls: Total worm burdens (healthy worms) will remain constant throughout the analysis period (30% - 60% of cercarial exposure dose). There will be no or very few dead or abnormally developed worms present.

2) Infected, Niridazole-treated (160 mgs/kg/day X 5 days): There will be an increase in dead worm burden with time after treatment, from no or few dead worms on Day 3 post-treatment to greater than 90% dead worms on days 13 and 20 post-treatment. However, the total worm burden (dead + living worms) will remain high throughout the analysis period (Days 3 - 20 post-treatment).

3) Infected, treated with Oxamniquine (100 mgs/kg/day X 5 days): Greater than 90% of the worm burden will be killed by Day 3 post-treatment. The majority of the dead worms will be in the liver and will have undergone considerable deterioration (with concurrent dead worm granuloma formation) by day 20 post-treatment.

4. Results of Drug Testing. Tables 3 through 8 represent all drug testing results. A summarization of these results indicates that of the 591 PMT and 474 PCT compounds tested, 152 (25%) were PMT retests of previously tested drugs and 33 (7%) were PCT retests. Additionally 87 (15%) PMT compounds and 44 (9%) PCT compounds were tested twice during the reporting period because of toxicity or unconfirmed activity. Tables 3 and 6 identify those compounds which were reported as unconfirmed or confirmed actives in their respective tests. In the PMT, 18 compounds were reported as such (Table 3). All but three of these were in dose response tests, and all represent retests of compounds confirmed as active in previous years. Many of them, however, are now reported active in a wider range of dosages than previously reported. In the PCT, 40 compounds were reported as confirmed or unconfirmed actives (Table 6). Of these, 7 compounds represent PCT retests from previous years, 6 of which were tested under dose response conditions (see PMT tests above). All of the remaining 33 compounds were tested for the first time in FY79.

In the identification of active compounds, primary reference is made only to the bottle code numbers (and corresponding Brazil numbers) since many (but not all) of the compounds that we receive are protected proprietary secrets ("commercially discreet"). We

have, however, identified below the general classes of the more significant non-discreet active compounds. Numbers in parentheses represent the number of compounds of each class which showed indications of activity. For the PMT these are:

heavy metals (5)
quinoline methanols (3)
nitro vinyl furan (1)
nitro diphenyl isotheocyonate (1)

For the PCT these are:

acridine (3)
nitrofuran (2)
heavy metals (2)
piperazine (1)
fluorene methanol (1)
8-aminoquinoline (1)
quinoline methanol (1)
phenanthrene methanol (1)

TABLE 2

Proposed protocol for the performance of the Secondary Curative Test (SCT) in the anti-schistosome drug development program of USAMRU-Brasilia.

Day	Procedure
0	Exposure all but 10 mice to 80-100 SmC
33	Divide all mice into the following groups: Uninfected/No Rx ..... 10 mice Infected/No Rx ..... 80 mice Infected/Rx Nirid. 160 ... 40 mice Infected/Rx Oxam 100 ..... 40 mice Infected/Rx Exp. Drug .... (40) mice/drug/dose/route*
33-37	Treat all mice (x mg/kg/day X 5 days)
40	Sacrifice ¼ of each group (Day 3 post-Rx)**
43	Sacrifice ¼ of each group (Day 6 post-Rx)**
50	Sacrifice ¼ of each group (Day 13 post-Rx)**
57	Sacrifice ¼ of each group (Day 20 post-Rx)**

*
The number of mice per test drug is to be determined on a drug-by-drug basis and is dependent upon the quantity of drug available. Under normal conditions use 40 mice per test drug group if sufficient drug quantities are available. Run as many experimental drug groups as the supply of mice permits.

**
Each mouse is to be killed by heparinized sodium pentabarbitol, perfused and "liver pressed". Total worm burden will be recorded as "Living" and "Dead" and will be expressed as the sum of those perfused and those observed in the liver. Worms recovered will be killed, fixed and preserved in AFA for further analysis.

TABLE 3

Compounds examined in the Primary Mortality Test System (PMTS) against S. mansoni during FY79 and reported active at the test dosages indicated. The reported resu. in parentheses represents the number of mice surviving 14 days or more after the mean day of death of the untreated control mice compared to the total number of mice in the drug test group. An "unconfirmed" result represents activity in an initial test at the indicated dosage/route of administration but confirmation at the same dosage/route of administration has not yet been accomplished. "Test Run" is the Julian date on which the testing was initiated by mouse exposure to 3000 or more cercariae.

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Surviving Mice)	Confirmation
00010	AG68873 =ZM27783	78339	160	SQ	Inactive (1/10)	NA
			320	SQ	Inactive (1/10)	NA
			640	SQ	Inactive (1/10)	NA
			1280	SQ	Active (5/10)	Unconfirmed
			1920	SQ	Active (3/5)	Confirms PMT 74289
			160	Gavage	Inactive (0/10)	NA
			320	Gavage	Inactive (1/10)	NA
			640	Gavage	Inactive (0/10)	NA
			1280	Gavage	Inactive (1/10)	NA
00012 =00014 =00017	AY29559 =AY29568 =BB21481	78339	20	SQ	Inactive (0/5)	NA
			40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (0/5)	NA
			160	SQ	Active (5/5)	Unconfirmed
			320	SQ	Active (5/5)	Unconfirmed (Prior confirmed Active at 640 and 1920 mg/kg)

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Surviving Mice)	Confirmation
00012	AY29559	78339	20	Gavage	Inactive (0/5)	NA
=00014	=AY29568		40	Gavage	Inactive (0/5)	NA
=00017	=BB21481		80	Gavage	Inactive (0/5)	NA
			320	Gavage	Inactive (1/5)	NA
			640	Gavage	Inactive (0/5)	NA
00121	BB57310	78347	80	SQ	Active (3/5)	Unconfirmed (Prior confirmed Active at 640 and 1920 mg/kg)
01312	BE21931	78326	160	SQ	Inactive (0/5)	NA
=04677			320	SQ	Inactive (0/5)	NA
			640	SQ	Inactive (1/5)	Confirmed Active in FY78
			1280	SQ	Active (4/5)	Unconfirmed
			160	Gavage	Inactive (0/5)	NA
			320	Gavage	Inactive (1/5)	NA
			640	Gavage	Inactive (0/5)	NA

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Surviving Mice)	Confirmation
01313	BE21968	78347	20	SQ	Inactive (0/5)	NA
			40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (0/5)	NA
			160	SQ	Active (4/5)	Confirmed
			320	SQ	Active (5/5)	Confirmed
			640	SQ	Active (5/5)	Confirmed
			20	Gavage	Inactive (1/5)	NA
			40	Gavage	Inactive (1/5)	NA
			80	Gavage	Inactive (0/5)	NA
			160	Gavage	Inactive (0/5)	NA
			320	Gavage	Inactive (1/5)	NA
			640	Gavage	Active (4/5)	Unconfirmed
			1280	Gavage	Active (5/5)	Unconfirmed
01317	AJ57633 =ZM33505	78354	160	SQ	Inactive (0/5)	NA
			320	SQ	Inactive (1/5)	NA
			640	SQ	Active (5/5)	Confirms PMT 76091 and PMT 75064 (prior report)
			160	Gavage	Inactive (0/5)	NA
			320	Gavage	Inactive (0/5)	NA
			640	Gavage	Inactive (1/5)	NA

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Surviving Mice)	Confirmation
01368 =04357	BE19575	78326	80	SQ	Inactive (1/5)	NA
			160	SQ	Active (4/5)	Confirms PMT 76217
			320	SQ	Toxic	NA
01567	BE13813 =ZN37106	78333	1280	SQ	Active (3/5)	Unconfirmed
01626 =04502	BG41577	78354	20	SQ	Inactive (0/5)	NA
			40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (0/5)	NA
			160	SQ	Active (5/5)	Unconfirmed
			320	SQ	Active (5/5)	Unconfirmed
			640	SQ	Active (3/5)	Unconfirmed
			1280	SQ	Active (5/5)	Unconfirmed
			20	Gavage	Inactive	NA
			40	Gavage	Inactive	NA
			80	Gavage	Inactive	NA
			160	Gavage	Inactive	NA
			320	Gavage	Inactive	NA
			640	Gavage	Inactive	NA
01674	BG43731 =ZN31953 =ZN80572	78347	1280	SQ	Active (4/5)	Confirms PMT 76126

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Surviving Mice)	Confirmation
02560	AX94257	78339	5	SQ	Inactive (1/5)	NA
=02909	=BH58880		10	SQ	Inactive (0/5)	NA
=04709	=ZN07500		20	SQ	Inactive (0/5)	NA
			40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (1/5)	NA
			160	SQ	Active (5/5)	Confirms PMT 78116 and PMT 77145
			40	Gavage	Inactive (1/5)	NA
			160	Gavage	Active (4/5)	Unconfirmed
			640	Gavage	Active (5/5)	Unconfirmed
02889	BH08111	79005	40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (0/5)	NA
			160	SQ	Inactive (1/5)	NA
			320	SQ	Inactive (2/5)	NA
			640	SQ	Inactive (1/5)	Confirmed Active in FY78
			1280	SQ	Active (4/5	Unconfirmed

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Surviving Mice)	Confirmation
02889	BH08111	79005	40	Gavage	Inactive	NA
			80	Gavage	Inactive	NA
			160	Gavage	Inactive	NA
			320	Gavage	Inactive	NA
			640	Gavage	Inactive	NA
			1280	Gavage	Inactive	NA
02893	BH09157	79024	40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (2/5)	NA
			160	SQ	Active (4/5)	Unconfirmed
			640	SQ	Not Done	Confirmed Active in FY78
			40	Gavage	Inactive	NA
			80	Gavage	Inactive	NA
			160	Gavage	Inactive	NA
02894	BH08166	79024	40	SQ	Inactive (1/5)	NA
			80	SQ	Active (3/5)	Unconfirmed
			160	SQ	Inactive (1/5)	NA
			320	SQ	Active (4/5)	Unconfirmed
			640	SQ	Not Done	Confirmed Active in FY78

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Surviving Mice)	Confirmation
02894	BH08166	79024	40	Gavage	Inactive	NA
			80	Gavage	Inactive	NA
			160	Gavage	Inactive	NA
			320	Gavage	Inactive	NA
02897	BH08200	79010	40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (1/5)	NA
			160	SQ	Inactive (2/5)	NA
			320	SQ	Active (3/5)	Unconfirmed
			640	SQ	Inactive (2/5)	Confirmed Active in FY78
			1280	SQ	Active (4/5)	Unconfirmed
			40	Gavage	Inactive (1/5)	NA
			80	Gavage	Inactive (0/5)	NA
			160	Gavage	Inactive (0/5)	NA
			320	Gavage	Inactive (0/5)	NA
			640	Gavage	Inactive (0/5)	NA
			1280	Gavage	Inactive (0/5)	NA

TABLE 3 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Surviving Mice)	Confirmation
02899	BH08228	79010	40	SQ	Inactive (0/5)	NA
			80	SQ	Inactive (0/5)	NA
			160	SQ	Inactive (1/5)	NA
			320	SQ	Active (4/5)	Unconfirmed
			640	SQ	Inactive (2/5)	Confirmed Active in FY78
			1280	SQ	Active (5/5)	Unconfirmed
			40	Gavage	Inactive	NA
			80	Gavage	Inactive	NA
			160	Gavage	Inactive	NA
			320	Gavage	Inactive	NA
			640	Gavage	Inactive	NA
			1280	Gavage	Inactive	NA
03809	BH30033	79017	1280	SQ	Not Done	Confirmed Active in FY78
			10	Gavage	Inactive (0/5)	NA
			40	Gavage	Inactive (0/5)	NA
			80	Gavage	Inactive (2/5)	NA
			160	Gavage	Inactive (1/5)	NA
			320	Gavage	Inactive (1/5)	NA
			640	Gavage	Active (5/5)	Unconfirmed
			1280	Gavage	Active (3/5)	Unconfirmed

TABLE 4

Compounds screened in the Primary Mortality Test System (PMTS) against S. mansoni during FY79 and determined to be toxic (T) at the test dosages indicated. Repetition of the same dose indicates that the compound was retested for confirmation. The lack of a toxicity indicator (T) represents non-toxicity and inactivity at that dosage. All drugs were administered subcutaneously.

Brazil Number	Bottle Number	Dosage (mg/kg)
00315	BB71132	640 T
00516	BC21600	40
		80
		160 T
00959	BB91563	20
		40
		80
		160
		320 T
00962	BB91616	120
		320
		640 T
00116	BE43786	320 T
01334	BE67651	640 T
01432	BE70881	1280 T
01461	BE67571	1280 T
01525	BB43996	1280 T
01818	BG43937	40
		80
		160 T
02040	BG47775	1280 T
02157	BG60189	40 T
02245	BG60161	40 T
02246	BG62807	40 T
02307	BE98076	80 T
02421	BG69100	160 T
		320 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
02452	BG72590	40 T
02470	BG80574	40 T
02923	BH09323	20 T
02924	BH09332	20 T
02963	BH12964	20 T
02990	BH14360	160 T
03038	AT90777	640 1280 T
03066	BH13916	640 T
03069	BH16613	160 T
03070	BH16711	160 T
03199	BC39344	320 T 640 T
03204	BC39620	320 640 T
03206	BC39853	320 T 640 T
03208	BC39979	320 T 640 T
03209	BC45904	320 T 640 T
03226	BB65607	320 640 T
03228	BC10509	320 T 640 T
03229	BC11239	320 T 640 T
03230	BC13073	320 T 640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03235	BC30690	320 T 640 T
03236	BC30805	640 T
03240	BC31615	640 T
03241	BC31928	640 T
03243	BC32078	640 T
03244	BC32176	640 T
03246	BC32498	640 T
03248	BC32532	640 T
03249	BC32578	640 T
03250	BC32587	640 T
03251	BC32701	640 T
03252	BC32890	160 640 T
03256	BC35220	160 640 T
03260	BC39139	160 T 640 T
03267	BE39755	160 640 T
03273	BB66051	160 640 T
03274	BB66113	160 T 640 T
03276	BC11328	160 640 T
03277	BC14794	160 T 640 T 640 T
03279	BC15577	160 640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03280	BC16118	160 640 T
03282	BC30627	160 240 T
03287	BC31777	160 640 T
03289	BC31964	160 T 640 T
03293	BC32096	320 640 T
03296	BC32185	80 640 T
03299	BC32541	80 640 T
03300	BC32550	80 640 T
03302	BC32765	80 640 T
03304	BC33299	80 640 T
03305	BC33404	160 640 T
03307	BC33477	80 640 T
03308	BC33486	80 640 T
03310	BC34063	80 640 T
03311	BC34072	80 640 T
03312	BC34090	80 640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03313	BC34125	80 640 T
03318	BH23930	80 640 T
03319	BH23949	80 640 T
03320	BH23958	80 640 T
03321	BH23967	80 640 T
03323	BH23985	80 640 T
03325	BH24008	80 640 T
03326	BH24017	80 640 T
03327	BC34778	80 T 640 T
03329	BC35426	80 640 T
03331	BC35855	80 640 T
03334	BC36521	86 640 T
03336	BC79066	80 T 640 T
03337	BC37608	80 T 640 T
03338	BC37751	80 T 640 T
03341	BC38105	80 T 640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03342	BC38123	80 T 640 T
03343	BC38392	80 640 T
03344	BC38918	80 T 640 T
03348	BC63779	160 640 T
03349	BD85056	80 640 T
03350	BD85501	80 640 T
03354	BD99069	160 640 T
03356	BE64230	160 640 T
03370	BH17218	80 T 640 T
03372	BH17236	80 T 640 T
03373	BH17245	80 T 640 T
03375	BB66211	320 640 T
03378	BC10189	320 640 T
03380	BC10483	320 640 T
03382	BC16252	160 T 640 T
03386	BC31099	320 T 640 T 640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03397	BC33217	320 640 T
03398	BC33459	160 T 640 T
03400	BC33619	160 640 T
03401	BC33708	320 640 T
03404	BC34643	160 640 T
03406	BC35033	320 640 T
03408	BC35837	160 T 640 T
03409	BC36692	160 640 T
03414	BC39442	160 640 T
03415	BC39602	160 640 T
03416	BC39960	320 640 T
03421	BD93996	160 T 640 T
03426	BB65483	160 640 T
03434	BC10821	160 T 640 T
03440	BC11060	640 T
03447	BC11266	640 T
03453	BC11524	640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03466	BC12030	640 T	03551	BG19066	640 T
03479	BC12281	640 T	03552	BH29852	640 T
03489	BC15540	640 T	03553	BH29898	640 T
03494	BC31053	640 T	03560	BG10223	640 T
03500	BC34625	640 T	03561	BG10581	640 T
03506	BH17281	640 T	03565	BG16430	640 T
03517	BC11140	640 T	03566	BG16565	640 T
03522	BC11480	640 T	03574	BG17820	640 T
03537	BC11818	640 T	03577	BG17868	640 T
03542	BG12405	640 T	03578	BG18005	640 T
03543	BG12441	640 T	03580	BG18578	640 T
03548	BG15433	640 T	03581	BG18738	640 T
03550	BG18649	640 T	03582	BG18818	640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03584	BG18836	640 T	03604	BC38249	640 T
03585	BG18907	640 T	03608	AY99588	640 T
03586	BG18952	640 T	03610	BB41830	640 T
03588	BG19075	640 T	03611	BB41849	640 T
03589	BC19128	640 T	03632	BC36825	640 T
03590	BC19637	640 T	03636	BC10198	640 T
03591	BC19646	640 T	03637	BC10250	640 T
03592	BC19655	640 T	03640	BC10385	640 T
03593	BC19682	640 T	03647	BC10607	640 T
03595	BC19708	640 T	03649	BC10689	640 T
03599	BC33262	640 T	03651	BC10705	640 T
03600	BC34803	640 T	03652	BC10750	640 T
03601	PC36165	640 T	03665	BC13224	640 T

TABLE 4 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
04405	BG81679	640 1280 T

TABLE 5

Compounds screened in the Primary Mortality Test System (PMTS) against S. mansoni during FY79 and determined to be inactive and non-toxic at the test dosages indicated. All compounds were administered subcutaneously.

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
00033	BB89821	160	00334	BB71534	640
00206	BB67414	160	00336	BB71561	640
00306	BB70948	640	00337	BB71570	640
00307	BB70957	640	00338	BB71598	640
00310	BB71061	640	00340	BB71641	640
00318	BB71187	640	00341	BB71669	640
00322	BB71258	640	00345	BB88708	640
00328	BB71409	640	00346	BB88717	640
00329	BB71418	640	00348	BB88735	640
00332	BB71454	640	00349	BB88744	320
00333	BB71507	640	00356	BB88851	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
00359	BB88888	160
00361	BB88922	640
00363	BB89009	640 1280 1920
00365	BB70822	160
00424	BE15148	640
00563	BC21628	640
00625	BD68340	640
00631	BB44484	640
00782	BC21253	640
00821	BC26794	640
00891	BB74320	80 160
00923	BB88093	320
00935	BB88280	640
01011	BB92695	160 320
01021	BE17615	1280
01118	BE43802	160
01178	BB47725	1280
01183	BB47903	1280
01215	BC52874	1280
01266	AV13275	320
01321	BE57646	80 160 320

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
01336	BE67679	40
		80
		160
01340	BE67731	1280
01387	BE70390	1280
01392	BE70470	1280
01394	BE70505	1280
01407	BE70783	1280
01408	BE70792	1280
01462	BE70658	1280
01489	BE18710	1280
01491	BE18747	1280
01493	BE18774	1280
01519	BG09284	1280
01520	BG09293	1280
01628	BG44014	1280
01630	AY98670	640
		1280
		1920
01681	AV99065	1280
01698	BE97211	1280
01701	BE97300	1280
01714	BG41086	80
		160
		320
01721	BE97426	1280
01816	BG39684	40
		80
		160
01865	BE97891	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
01883	BG39693	320
02040	BG47686	80 160 320
02054	BG47855	1280
02263	BG68443	40
02266	BG70729	40
02267	BG70756	40
02281	AY46050	80
02301	BE93017	40
02302	BE97837	40
02304	BE97926	320
02312	BE98192	40
02324	AG56396	40
02329	AK23525	80
02372	BG39415	80
02379	BG39997	80
02393	BG40963	80
02396	BG58689	80
02400	BG59248	40
02426	BG69431	80
02433	BG69860	40
02445	AV37127	160 320
02447	BC82023	40
02456	BG75064	1280
02471	BG80618	80

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
02472	BG80672	40
02492	BG81124	1280
02664	BC15273	1280
02672	BC15460	640 1280
02844	BH05727	40
02865	BH09387	20
02872	BH07936	40
02874	BH07954	20
02875	BH07963	20
02876	BH07972	40
02877	BH07981	40
02878	BH07990	20
02900	BH08237	20
02901	BH08246	40
02911	BH09181	640
02918	BH09261	20
02919	BH09270	40
02922	BH09314	40
02951	BH10317	20
02956	BH09921	20
02959	BH09985	20
02996	AF92511	320
03006	AV58373	160
03016	AE07642	160
03048	AX29054	160

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03052	AY72309	160
03053	AY72407	160
03174	AU65162	640 960
03177	AU65966	640 960
03194	BC39228	640
03195	BC39282	640
03196	BC39291	640
03197	BC39308	640
03198	BC39326	640
03200	BC39522	640
03201	BC39531	640
03202	BC39540	640
03203	BC39611	640
03205	BC39808	640
03207	BC39899	640
03210	BD52486	640
03211	BE82621	640
03212	BE98156	640
03213	BH16560	640
03219	ZM34333	640
03220	BH16677	640
03221	BH16686	640
03222	BH16695	640
03223	BH16702	640
03224	BH16828	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03225	BB65410	640	03257	BC35239	640
03227	BC10134	640	03258	BC36530	640
03232	BC16261	640	03259	BC37895	640
03233	BC16298	640	03261	BC39193	640
03234	BC30645	640	03262	BC39424	640
03237	BC30841	640	03263	BC39764	640
03238	BC30850	640	03264	BC39773	640
03239	BC31446	640	03265	BD85216	640
03242	BC32041	640	03266	BD88762	640
03245	BC32363	640	03268	BE40294	640
03253	BC32916	640	03269	BE64221	640
03254	BC33771	640	03270	BE64605	640
03255	BC34714	640	03271	BE64758	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03272	BB65714	640
03275	BC10867	640
03278	BC15424	640
03281	BC30038	640
03283	BC31035	640
03284	BC31400	640
03285	BC31633	640
03286	BC31713	640
03288	BC31919	640
03290	BC31973	640
03291	BC31982	640
03292	BC31991	640
03294	BC32112	640
03295	BC32130	80 640
03297	BC32194	640
03298	BC32201	640
03301	BC32685	640
03303	BC32907	640
03306	BC33440	640
03309	BC33566	640
03314	BC34563	640
03315	BC34572	640
03316	BC34705	640
03317	BC23921	640
03322	BH23976	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03324	BH23994	640	03353	BD91821	640
03328	BC35248	640	03355	BE39442	640
03330	BC35499	640	03357	BH16971	640
03332	BC36138	640	03358	BH17030	640
03333	BC36370	640	03359	BH17058	640
03335	BC37117	640	03360	BH17067	640
03339	BC37902	640	03361	BH17076	640
03340	BC37966	640	03362	BH17094	640
03345	BC39077	640	03363	BH17101	640
03346	BC39120	640	03364	BH17110	640
03347	BC39826	640	03365	BH17129	640
03351	BD88735	640	03366	BH17138	640
03352	BD90235	640	03367	BH17147	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03368	BH17165	640
03369	BH17209	640
03371	BH17227	640
03374	BB65938	640
03376	BC10063	640
03377	BC10152	640
03379	BC10438	640
03381	BC16136	640
03383	BC19548	640
03384	BC30163	640 960
03385	BC30789	640
03387	BC31124	640
03388	BC31508	640
03389	BC32167	640
03390	BC32309	640
03391	BC32452	640
03392	BC32676	640
03393	BC32694	640 960
03394	BC32729	640
03395	BC32756	640
03396	BC32836	640
03399	BC33502	640
03402	BC33735	640
03403	BC33879	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03402	BC33735	640
03403	BC33879	640
03405	BC34750	640
03407	BC35202	640
03410	BC36709	640
03411	BC37555	640
03412	BC38767	640
03413	BC39157	640
03417	BC63537	640 960
03418	BC63715	640
03419	BD85903	640
03420	BD90691	640
03422	BH26986	640
03424	ZN37704	640
03425	BB62802	640 960
03427	BB65625	640
03428	BB65830	640
03429	BB65965	640
03430	BB65983	640
03431	BB66060	640
03432	BB66239	640
03433	BB91812	640
03435	BC10830	640
03436	BC10885	640
03437	BC10947	640
03438	BC10965	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03439	BC11006	640	03458	BC11640	640
03442	BC11159	640	03460	BC11702	640
03444	BC11195	640	03461	BC11837	640
03445	BC11202	640	03463	BC11917	640
03446	BC11257	640	03464	BC11935	640
03448	BC11300	640	03465	BC12003	640
03450	BC11355	640	03467	BC12058	640
03451	BC11471	640	03469	BC12110	640
03454	BC11533	640	03470	BC12129	640
03455	BC11542	640	03471	BC12138	640
03456	BC11597	640	03472	BC12156	640
03457	BC11631	640	03473	BC12165	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03474	BC12209	640	03492	BC15675	640
03475	BC12218	640	03493	BC15906	640
03476	BC12227	640	03495	BC32069	640
03477	BC12236	640	03496	BC33682	640
03478	BC12263	640	03497	BC33691	640
03480	BC12290	640	03498	BC33977	640
03481	BC12343	640	03499	BC43223	640
03485	BC15362	640	03501	BC38178	640
03486	BC15479	640	03502	BC39273	640
03487	BC15504	640	03503	BC63653	640
03488	BC15522	640	03504	BH17156	640
03490	BC15602	640	03505	BH17174	640
03491	BC15648	640	03507	ZN38489	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03508	ZN39262	640	03526	BC11588	640
03509	BH17049	640	03529	BC11677	640
03511	BC10849	640	03530	BC11686	640
03512	BC10901	640	03531	BC11720	640
03513	BC10929	640	03532	BC11739	640
03514	BC10974	640	03533	BC11748	640
03515	BC11015	640	03534	BC11766	640
03516	BC11122	640	03536	BC11800	640
03519	BC11280	640	03538	BC11828	640
03520	BC11364	640	03539	BC11891	640
03521	BC11444	640	03540	BC11908	640
03523	BC11499	640	03541	BC11980	640
03525	BC11560	640	03545	BG12478	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03546	BG15228	640	03568	BG17008	640
03547	BG15317	640	03569	BG17133	640
03549	BG15835	640	03570	BG17320	640
03554	BH29914	640	03571	BG17508	640
03555	BH29941	640	03572	BG17553	640
03556	BH29950	640	03573	BG17562	640
03557	BH29987	640	03575	BG17839	640
03558	BH30346	160	03576	BG17857	640
03559	BH30355	160	03579	BG18470	640
03562	BG12254	640	03583	BG18827	640
03563	BG14203	640	03587	BC19039	640
03564	BG15353	640	03594	BC19691	640
03567	BG16994	640	03597	BC30029	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
03598	BC31179	640			
03602	BC36245	640	03642	BC10518	640
03603	BC36496	640	03643	BC10536	640
03605	BC38365	640	03644	BC10554	640
03606	BC39513	640	03645	BC10563	640
03607	BC39791	640	03646	BC10572	640
03609	BB40422	640	03648	BC10616	640
03631	BC35711	640	03650	BC10698	640
03633	BC10090	640	03653	BC10803	640
03634	BC10107	640	03654	BC10983	640
03638	BC10269	640	03656	BC11051	640
03639	BC10278	640	03657	BC11113	640
03641	BC10492	640	03658	BC11248	640

TABLE 5 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
03659	BC11319	640
03660	BC11346	640
03661	BC11373	640
03662	BC11426	640
03663	BC11579	640
03664	BC13162	640
04647	BH56608	320 640

TABLE 6

Compounds examined in the Primary Curative Test (PCT) System against S. mansoni during FY79 and reported active in one or more tests at the test dosages indicated. Numbers in parentheses represent the mean number of worms in the livers of surviving treated mice. A compound is considered active in the PCT if mean liver worm burdens reach 20 or more; a mean of 20 to 25 worms represents "marginal" (M) activity. An "unconfirmed" result represents activity in an initial tests at the indicated dosage/route of administration but confirmation at the same dosage/route of administration had not yet been accomplished. "Test run" is the Julian date on which the testing was initiated by mouse exposure to approximately 200 (± 10%) cercariae.

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
01179	BB47734	79031	160	Gavage	Active (28)	Confirms PCT 76329
			320	Gavage	Toxic	NA
01203	BC07271	79031	40	Gavage	Inactive (12)	NA
=04666			80	Gavage	Inactive (14)	NA
			160	Gavage	Inactive (19)	NA
			320	Gavage	Active (50)	Confirms PCT 78144
			640	Gavage	Active (45)	Unconfirmed
01533	BC42725	79031	5	SQ	Inactive (17)	NA
=05104	=BH73209		10	SQ	Inactive (17)	NA
			20	SQ	Active (21)(M)	Unconfirmed
			40	SQ	Not Done	Confirmed Active in FY78

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (JUlian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
01617	BG32514	79031	10	SQ	Inactive (15)	NA
			20	SQ	Active (22)(M)	Unconfirmed
			40	SQ	Toxic	NA
02456	BG75064	79031	80	Gavage	Active (33)	Unconfirmed Prior confirmed activity at 100 and 200 mg/kg (SQ) reported in FY78
02457	BG75073	79031	80	Gavage	Active (23)(M)	Unconfirmed
			160	Gavage	Active (40)	Unconfirmed
02685	BB69329	79031	5	SQ	Inactive (13)	NA
=03508	ZN39262		10	SQ	Inactive (15)	NA
			20	SQ	Inactive (18)	NA
			40	SQ	Active (26)	Confirmed
			80	SQ	Active (33)	Unconfirmed
			160	SQ	Active (17; 20% dead)	Unconfirmed
			5	Gavage	Inactive (15)	NA
			10	Gavage	Toxic	NA
			20	Gavage	Inactive (16)	NA
			40	Gavage	Inactive (16)	NA
			80	Gavage	Active (27)	Unconfirmed
			160	Gavage	Active (29)	Unconfirmed

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
02882	BH08040	79031	40	SQ	Active (19, 90% with abnormal development)	Unconfirmed
04800	AB10813	78277	100	SQ	Active (21)(M)	Unconfirmed (marginal)
		79031	80	SQ	Inactive (10)	NA
			160	SQ	Inactive (10)	NA
04804	AB16253	78277	100	SQ	Active (20)(M)	Unconfirmed (marginal)
		79031	80	SQ	Inactive (8)	NA
			100	SQ	Inactive (7)	Fails to confirm marginal activity in 78277
04807	AB27363	78277	100	SQ	Toxic	NA
		79045	40	SQ	Active (22)(M)	Unconfirmed
			80	SQ	Toxic	NA
		79143	20	SQ	Inactive (10)	NA
			40	SQ	Inactive (11)	Fails to confirm activity in PCT 79045. Testing terminated.

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
04813	AB55769	78277	100	SQ	Active (20)(M)	Unconfirmed
		79031	160	SQ	Inactive (13)	Fails to confirm activity at a lower dose in PCT 78277. Testing terminated.
04833	AB88777	79052	100	SQ	Active (20)(M)	Unconfirmed
		79122	50	SQ	Inactive (18)	NA
			100	SQ	Active (20)(M)	Confirms marginal activity of PCT 79052
04899	AD44953	79122	100	SQ	Active (20)(M)	Unconfirmed
		79157	100	SQ	Inactive (15)	Fails to confirm marginal activity of PCT 79122. Testing terminated.
04900	AD45772	79073	100	SQ	Toxic	NA

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
04900	AD45772	79122	50	SQ	Toxic	NA
		79157	10	SQ	Inactive (11)	NA
			20	SQ	Active (26)	Unconfirmed
04939	AE00634	79087	100	SQ	Toxic	NA
		79122	50	SQ	Toxic	NA
		79157	10	SQ	Active (20)(M)	Unconfirmed
			20	SQ	Active (21)(M)	Unconfirmed
04954	AE12769	79101	100	SQ	Active (27)	Unconfirmed
		79122	50	SQ	Inactive (18)	NA
			100	SQ	Active (20)(M)	Confirms PCT 78101
		79157	80	SQ	Active (24)(M)	Unconfirmed
04955	AE14389	79101	100	SQ	Active (22)(M)	Unconfirmed
		79122	50	SQ	Inactive (17)	NA
			100	SQ	Inactive (16)	Fails to confirm marginal activity of PCT 79101. Testing terminated.

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
04970	AE43522	79101	100	SQ	Active (21)(M)	Unconfirmed
05044	AD08402	79108	100	SQ	Active (22)(M)	Unconfirmed
05068	AD32695	79122	100	SQ	Active (20)(M)	Unconfirmed
		79157	100	SQ	Inactive (17)	Fails to confirm marginal activity in 79122. Terminate testing.
05070	AD33996	79122	100	SQ	Active (21)(M)	Unconfirmed
		79157	100	SQ	Inactive (18)	Fails confirm marginal activity in PCT 79122.
05122	BH73449	79157	100	SQ	Active (23)(M)	Unconfirmed
05133	BH50099	79171	100	SQ	Active (51)	Unconfirmed
05134	BH67549	79171	100	SQ	Active (23)(M)	Unconfirmed
05136	BH73216	79171	100	SQ	Active (23)(M)	Unconfirmed

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Results (Mean No. Worms/Liver)	Confirmation
05180	AF50488	79171	100	SQ	Active (23)(M)	Unconfirmed
05190	AT11169	79171	100	SQ	Active (28)	Unconfirmed
05191	AT13518	79171	100	SQ	Active (36)	Unconfirmed
05194	AT16897	79171	100	SQ	Active (25)	Unconfirmed
05198	AT27194	79171	100	SQ	Active (20)(M)	Unconfirmed
05199	AT27738	79171	100	SQ	Active (22)(M)	Unconfirmed
05209	AT33665	79185	100	SQ	Active (21)(M)	Unconfirmed
05210	AT33852	79185	100	SQ	Active (23)(M)	Unconfirmed
05214	AT48559	79185	100	SQ	Active (23)(M)	Unconfirmed
05229	AT70337	79185	100	SQ	Active (20)(M)	Unconfirmed
05232	AT71414	79185	100	SQ	Active (20)(M)	Unconfirmed
05622	BJ08205	79122	100	SQ	Toxic	NA

TABLE 6 (continued)

Brazil Number	Bottle Number	FY79 Test Run (Julian Date)	Drug Administration Dosage (mg/kg)	Drug Administration Route	Reported Test Result (Mean No. Worms/Liver)	Confirmation
05622	BJ08205	79157	10	SQ	Inactive (17)	NA
			20	SQ	Active (22)(M)	Confirmed
			40	SQ	Toxic	NA

TABLE 7

Compounds screened in the Primary Curative Test (PCT) system against S. mansoni during FY79 and determined to be toxic (T) at the test dosages indicated. Repetition of the same dose indicates that the compound was retested for confirmation. The lack of a toxicity indicator (T) represents non-toxicity and inactivity at that dosage. All compounds were administered subcutaneously.

Brazil Number	Bottle Number	Dosage (mg/kg)
02873	BH07945	40 T
02902	BH08255	40 T
04582	BE19397	50 T 100 T
04697	BH57865	50 100 T
04724	AC29826	50 T 100 T
04731	AC74956	50 100 T
04732	AC75088	10 50 T 100 T
04743	AD02268	10 50 T 100 T
04750	AD03194	50 100 T
04756	AD03667	10 50 T 100 T
04757	AD03685	10 50 T 100 T
04771	AD38419	50 T 100 T

TABLE 7 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
04796	AB09169	50
		100
		100 T
04825	AB81401	10
		50 T
		100 T
04835	AB91470	50
		100 T
04836	AB92799	10
		20
		50 T
		100 T
04842	AB92721	50
		100 T
04843	AC00090	50
		100 T
04845	AC01999	50
		100 T
04846	AC02754	50 T
		100 T
04852	AC13604	50
		100 T
04885	AD49761	50
		100 T
04890	AC96345	100 T
04938	AE00170	10
		20
		50 T
		100 T
04943	AE02405	10
		20
		50 T
		100 T
04946	AE16294	10
		20 T
		50 T
		100 T

TABLE 7 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
04958	AE27377	10
		20
		50 T
		100 T
04966	AE35735	50
		100 T
04972	AE48509	50
		100 T
04997	AV36620	10 T
		20 T
		50 T
		100 T
4998	AV36639	10
		20 T
		50 T
		100 T
04999	AV36728	10
		20
		50 T
		100 T
05000	AV36997	100 T
05001	AV37136	50 T
		100 T
05002	AC37145	50 T
		100 T
05005	AV37314	50 T
		100 T
05007	AV37458	50 T
		100 T
05014	BH65910	50
		100 T
05043	AD06113	50
		100 T
05055	AD16717	50
		100 T
05083	AD37752	100 T

TABLE 7 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
05109	BH70126	100 T
05116	BH73252	100 T
05123	BH73458	100 T
05139	BH67503	100 T
05142	BH72611	100 T
05187	AF89667	100 T
05188	AS00465	100 T
05192	AT13912	100 T
05193	AT13949	100 T
05207	AT31607	100 T
05208	AT33521	100 T
05228	AT70097	100 T
05248	AT78217	100 T
05261	AT85838	100 T
05265	AT86086	100 T
05268	AT88213	100 T
05269	BH81825	100 T
05271	AS03626	100 T

TABLE 8

Compounds screened in the Primary Curative Test (PCT) System against S. mansoni during FY79 and determined to be inactive and non-toxic at the test dosages indicated. All compounds were administered subcutaneously unless otherwise indicated (Gav = oral administration by gavage). A test dosage appearing twice for the same compound represents a retest at that dosage.

Brazil Number	Bottle Number	Dosage (mg/kg)
01011	BB92695	40 Gav
02890	BH08120	40
04587	AB60519	160
04701	BH57936	160
04754	AD03603	50 100
04779	AH61294	50 100
04788	BH58979	100
04789	BH58988	100
04793	AB02160	100
04794	AB06462	100
04795	AB07414	100
04797	AB09374	100
04798	AB09927	100
04799	AB09981	100
04801	AB11785	100 100
04802	AB13298	100
04803	AB15578	100 100
04805	AB16557	100
04806	AB18775	100
04808	AB31027	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
04809	AB46064	100
04810	AB47061	100
04811	AB47187	100
04812	AB53390	100
04814	AB61267	100
04815	AB67278	100
04816	AB64429	100
04817	AB67536	100
04818	AB68462	100
04819	AB68864	100
04820	AB68908	100
04821	AB70640	100
04822	AB73294	100
04823	AB73678	100
04824	AB79394	100
04826	AB81689	100
04827	AB85687	100
04828	AB85696	100 100
04829	AB85749	100
04830	AB86309	100
04831	AB88633	100
04832	AB88697	160
04834	AB89265	100
04837	AB94551	100
04838	AB95496	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
04839	AB95503	100	04857	AC13917	100
04840	AB95781	100	04858	AC19311	100
04841	AB95807	100	04859	AC30703	100
04844	AC00287	100	04860	AC43317	100
04847	AC03591	100	04861	AC80098	100
04848	AC03635	100	04862	AC83786	100
04849	AC03831	100	04863	AC84596	100
04850	AC12438	100	04864	AC84612	100
04851	AC13597	100	04865	AD41630	100
04853	AC13819	100	04866	AD41658	100
04854	AC13846	100	04867	AD41809	100
04855	AC13882	100	04868	AD41818	100
04856	AC13891	100	04869	AD41925	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
04870	AD41952	100	04883	AD42879	100
04871	AD42388	100	04884	AD42931	100
04872	AD42397	100	04886	BG10358	100
04873	AD42413	100	04887	BG10438	100
04874	AD42422	100	04888	BG12147	100
04875	AD42431	100	04889	BH30784	100
04876	AD42459	100	04891	AD43901	100
04877	AD42468	100	04892	AD43992	100
04878	AD42486	100	04893	AD44006	100
04879	AD42673	100	04894	AD44015	100
04880	AD42682	100	04895	AD44033	100
04881	AD42708	100	04896	AD44042	100
04882	AD42842	100	04897	AD44051	100

TABLE 8 (continue)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
04898	AD44542	100	04913	AD52302	100
04901	AD45969	100	04914	AD53523	100
04902	AD45996	100	04915	AD53890	100
04903	AD47927	100	04916	AD70391	100
04904	AD48184	100	04917	AD71647	100
04905	AD48193	100	04918	AD72377	100
04906	AD48504	100	04919	AD73025	100
04907	AD48577	100	04920	AD73721	100
04908	AD48693	100	04921	AD74808	100
04909	AD48700	100	04922	AD76508	100
04910	AD49716	100	04923	AD77544	100
04911	AD49725	100	04924	AD77185	100
04912	AD50728	100	04925	AD77201	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
04926	AD77416	100	04941	AE00732	100
04927	AD77649	100	04942	AE02370	100
04928	AD77998	100	04944	AE03019	100
04929	AD78217	100	04945	AE03064	100
04930	AD84500	100	04946	AE03895	100
04931	AD85945	100	04947	AE04052	100
04932	AD86317	100	04948	AE07204	100
04933	AD86488	100	04949	AE07213	100
04934	AD86899	100	04950	AE07222	100
04935	AD87663	100	04951	AE07231	100
04936	AD87770	100	04952	AE12394	100
04937	AD87921	100	04953	AE12456	100
04940	AE00714	100	04959	AE27411	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
04960	AE28310	100	04976	AE49300	100
04961	AE29657	100	04977	AE49328	100
04962	AE30169	100	04978	AE53055	100
04963	AE31095	100	04979	AE56010	100
04964	AE35717	100	04980	AE56216	100
04965	AE35726	100	04981	AE56225	100
04967	AE38450	160	04982	AE56252	100
04968	AE38656	100	04983	AE56270	100
04969	AE43068	100	04984	AE58363	100
04971	AE48483	100	04985	AE59404	100
04973	AE49239	160	04986	AE73717	100
04974	AE49266	100	04987	AE75597	100
04975	AE49275	100	04988	AE83400	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
04989	AE84425	100
04990	AE86670	100 200
04991	AE86689	100
04992	AE96238	100
04993	AE14482	100
04994	AF52491	100
04995	AF55410	100
04996	AT14071	100
05003	AV37154	100
05004	AV37216	100
05006	AV37323	100
05008	AV38026	100
05009	AV55778	100
05010	AX52311	100
05011	AX52704	100
05012	BH65778	100
05013	BH65858	100
05015	BH65947	100
05016	BH65956	100
05017	BH65965	100
05018	BH65974	100
05019	BH66346	100
05020	BH66364	100
05021	BH66408	100
05022	BH66435	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05023	BH66444	100	05036	BH70162	100
05024	BH66480	100	05037	BH70171	100
05025	BH66499	100	05038	BH70180	100
05026	BH66631	100	05039	BH70199	100
05027	BH66757	100	05040	AD04468	100
05028	BH66819	100	05041	AD04511	100
05029	BH66828	100	05042	AD05661	100
05030	BH66873	100	05045	AD08457	100
05031	BH72353	100	05046	AD08466	100
05032	AF35963	100	05047	AD08939	100
05033	BH69945	100	05048	AD10359	100
05034	BH69990	100	05049	AD10420	100
05035	BH70144	100	05050	AD10528	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05051	AD10733	100	05065	AD28780	100
05052	AD10751	100	05066	AD29698	100
05053	AD10868	100	05067	AD29705	100
05054	AD13627	100	05069	AD32784	100
05056	AD18088	100	05071	AD34859	100
05057	AD18882	100	05072	AD35589	100
05058	AD20319	100	05073	AD36380	100
05059	AD21718	100	05074	AD36424	100
05060	AD23472	100	05075	AD36433	100
05061	AD26848	100	05076	AD36577	100
05062	AD28011	100	05077	AD36595	100
05063	AD28182	100	05078	AD36675	100
05064	AD28655	100	05079	AD36773	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05080	AD36906	100	05094	AD41694	100
05081	AD36915	100	05095	AD41854	100
05082	AD36960	100	05096	AD41907	100
05084	AD37921	100	05097	AD41970	100
05085	AD38277	100	05098	AD45227	100
05086	AD38795	100	05099	AD46779	100
05087	AD39176	100	05100	AD46966	100
05088	AD39578	100	05101	AD48326	100
05089	AD39667	100	05105	BH66551	100
05090	AD41185	100	05106	BH58951	100
05091	AD41274	100	05107	BH58997	100
05092	AD41283	100	05108	BH70108	100
05093	AD41569	100	05110	BH72915	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05111	BH72942	100	05128	BH73823	100
05112	BH72951	100	05130	BH16980	100
05113	BH72960	100	05132	BH50071	100
05114	BH72997	100	05135	BH67558	100
05115	BH73243	100	05140	BH72488	100
05117	BH65250	100	05141	BH72497	100
05118	BH67496	10^	05143	BH72666	100
05120	BH73421	100	05144	BH72817	100
05121	BH73430	100	05151	BH76253	100
05124	BH73467	100	05152	BH76262	100
05125	BH73494	100	05181	AF61589	100
05126	BH73501	100	05182	AF70355	100
05127	BH73510	100	05183	AF82328	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05184	AF86988	100	05206	AT31330	100
05185	AF88044	100	05211	AT34055	100
05186	AF88768	100	05212	AT34199	100
05189	AT10822	100	05213	AT48282	100
05195	AT18275	100	05215	AT48675	100
05196	AT25985	100	05216	AT48899	100
05197	AT26428	100	05217	AT49065	100
05200	AT28093	100	05218	AT57389	100
05201	AT28182	100	05219	AT58162	100
05202	AT28253	100	05220	AT63323	100
05203	AT28280	100	05221	AT63734	100
05204	AT30913	100	05222	AT64151	100
05205	AT30931	100	05223	AT64428	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)	Brazil Number	Bottle Number	Dosage (mg/kg)
05224	AT64704	100	05241	AT76802	100
05225	AT65130	100	05242	AT76893	100
05226	AT65596	100	05243	AT77210	100
05227	AT65658	100	05244	AT77283	100
05230	AT70542	100	05246	AT77729	100
05231	AT71325	100	05249	AT78511	100
05233	AT71781	100	05250	AT78708	100
05234	AT75056	100	05251	AT78940	100
05235	AT75298	100	05253	AT79367	100
05237	AT75458	100	05254	AT79590	100
05238	AT76615	100	05255	AT79670	100
05239	AT76624	100	05256	AT81027	100
05240	AT76795	100	05257	AT83183	100

TABLE 8 (continued)

Brazil Number	Bottle Number	Dosage (mg/kg)
05258	AT83272	100
05259	AT84840	100
05260	AT85829	100
05262	AT85981	100
05263	AT86022	100
05264	AT86040	100
05266	AT86219	100
05267	AT86728	100
05270	AS01524	100
05272	AS06814	100
05273	AS08541	100
05274	AS10649	100
05275	AS11851	100
05276	AS29068	100
05277	AS34587	100
05347	BH86464	40
05348	BH86473	40
05371	BH89536	40
05591	AT19058	100

PART II. CLINICAL, EPIDEMIOLOGICAL, IMMUNOLOGICAL AND ENTOMOLOGICAL STUDIES ON MALARIA IN AMAZONAS, BRAZIL, ALONG THE ITUXI RIVER.

1. General:

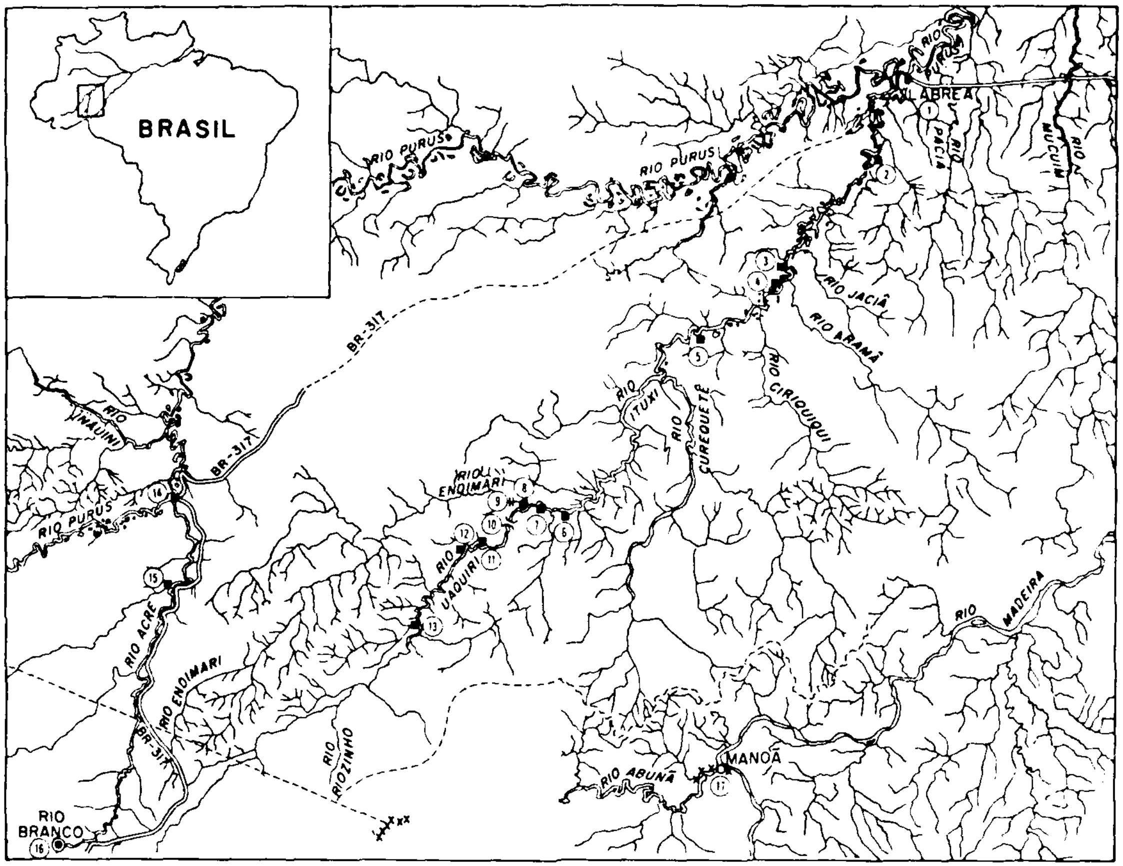
A program of studies on malaria in the Amazon Basin was initiated in CY 1978. The field site for these studies is along the Ituxi River, southwest of Labrea, Amazonas, Brazil (Figure 1). The operational headquarters and laboratories in the Núcleo de Medicina Tropical e Nutrição (Center of Tropical Medicine and Nutrition) at the University of Brasilia provide the logistical and technical support for our studies in the field. Over-all program objectives are to evaluate: a) the clinical and epidemiological aspects of malaria, b) the immunological aspects of malaria with increased emphasis on in vitro drug susceptibility testing, and c) the ecology and population dynamics of malaria vectors. The information from these, and associated, studies is fundamental to an understanding of the mechanisms affecting continued malaria transmission despite ongoing control measures in these areas.

2. Description of the Field Study Area:

The Ituxi River region is an excellent area for conducting studies on malaria ecology due to the existence of a high level of disease transmission in spite of active control efforts. The Ituxi is a branch river of the larger Purus River. The Ituxi-Purus confluence is located approximately 10 kilometers west of Labrea in Amazonas State. The Ituxi headwaters are found in the states of Acre and Amazonas. The terra firme and igapo habitats are the most frequently encountered habitats along the river (terra firme is highland that is never inundated by the river; igapo is composed of low areas that are inundated for several months each year). The Ituxi River residents comprise a widely distributed and stable community. They generally have been born and raised on this river system. Houses normally are built on terra firme and families earn their livelihood by collecting rocks for construction, rubber, latex and castanhas do Pará. Subsistence farming, hunting, and fishing are other principal activities.

3. Clinical and Epidemiological Studies of Malaria in Amazonas, Brazil:

a. Introduction: Although measures to eradicate malaria are applied throughout the state of Amazonas, the incidence and prevalence of this disease remains high. Malaria surveillance data obtained from Su-



CIDADES E VILAS

(1) LABREA	(7) SANÚACA	(13) BOCA DO RIOZINHO
(2) MISSÃO	(8) FORTALEZA DO ITUXI	(14) BOCADO ACRE
(3) PALMAPI	(9) CACHOEIRA DO MEIO (DEPÓSITO)	(15) FLORIANO PEIXOTO
(4) FLORESTA	(10) CACHOEIRA DA ÁGUA PRETA	(16) RIO BRANCO
(5) CAMARGO	(11) MATIPUÃ	(17) MANOÃ
(6) BACABAL	(12) BATATA	

Fig 1

perintendencia de Campanhas de Saũde Pũblica (SUCAM) reveal that Amazonas remains in the group of states within Brazil where malaria control is yet to be achieved. The slide positivity rates for these states exceeded those from the remainder of Brazil by approximately twenty-fold for each of the years 1974 through 1978 (Table 9). The average annual slide positivity rate for the municipality of Labrea, Amazonas, was 11.28% during this same period, although it appears that the disease may be receding somewhat in the urban areas (Table 10). These data evidence the continued significance of malaria in Brazil and serve as indicators of the amount of work yet to be done toward the eventual goal of eradication.

b. Objectives: The objectives of the clinical and epidemiological studies are to:

1) determine the influence of migration on the perpetuation of malaria endemicity;

2) establish the clinical events of malaria infections for future comparisons and evaluations;

3) determine the level of antimalarial antibodies in this population and correlate this with spleen sizes;

4) monitor boat traffic on the river to determine the impact of population movement on malaria endemicity.

c. Methods: Methods used to accomplish these objectives are:

1) accurate mapping of the study area and censusing to determine the number and location of residents along the river;

2) the initial clinical examination of at least 85-90% of the population with special interest in slide positivity rates and spleen size;

3) the collection of sera for antimalarial serological testing;

4) the performance of follow-up examinations and repeated testing.

d. Progress: The preliminary mapping and the census of the study area have been accomplished. Although the exact percentage of persons examined, as well as certain other clinical correlations, is unavailable due to incomplete computer analysis, it appears that the projected 85-90% of the population has been examined. The total study population consists of 155 families including 941 persons, or an average of 6.07 persons/family. A total of 1153

TABLE 9

Distribution of the index of malaria incidence in Brazil by slide positivity, 1974-1978, for all species of malaria (Source: SUCAM).

Geographical area of Brazil according to degree of malaria eradication	Percent Slide Positivity Rate				
	1974	1975	1976	1977	1978
Eradication nearing completion.....	0.7	0.7	0.5	0.5	0.5
Eradication only on a long-term basis..	9.8	10.1	10.5	10.5	10.9

TABLE 10

Malaria slide positivity rates for the municipality of Labrea, Amazonas, 1974-1978 for all species of malaria (Source: SUCAM).

Year	Number of Slides Examined	Number of Positives	Percent Positive
1974	2264	208	9.18
1975	1205	165	13.69
1976	1774	305	17.19
1977	856	73	8.52
1978	1841	145	7.87
Totals	7940	896	11.28

slides have been examined with a total of 90 positives (7.8%), 43 being Plasmodium falciparum and 47 being P. vivax. The age distribution of these perons with positive smears by species of malaria is given in Table 11.

A total of 1014 serum and filter paper samples have been tested from this population, although filter paper data gave sufficiently poor results to not be included in the analysis. Of all sera tested, 91.64% were positive for antimalarial IgG and 30.54% were positive for antimalaria IgM. The age distribution of persons with positive serologies is shown in Table 12. The low incidence of malaria antibodies, notably IgM, in the younger age groups is of interest because of the high rate of slide positivity in these same groups. One possible explanation that has been considered is that prompter chloroquine treatment of these younger individuals due to a more severe clinical course and earlier appearance of fever may result in decreased antibody production. The ubiquitous availability of chloroquine and the presumptive treatment of all fevers with this drug lend some credibility to this theory, but it is far from proven at this point and further investigation in needed.

Another interesting correlation exists between spleen rates and the serological data. Of 217 persons with a palpable spleen, 197 (90.78%) had significant levels of IgG and the remaining 20 persons without positive serologies were all less than 20 years of age. In the remaining group of 112 persons without a palpable spleen, 101 (90.18%) had positive IgG serologies and the 11 persons with negative serologies were again all under 20 years of age. These data indicate that serological screening for the presence of antimalarial IgG is a better measure of malaria prevalence than spleen surveys, certainly in persons over 20 years of age. It should also be noted that there was a much weaker correlation between spleen rates and the presence of antimalarial IgM. Further study of these relationships is desirable.

Table 13 presents comparison data between a group of 63 persons who had temporarily migrated into the interior of the jungle and 63 perons permanently residing on the river. Based on antimalarial IgM rates, the population remaining near the river appear to have a higher incidence of disease than the migrants, implicating areas nearer the river as being more active sites of transmission. Antimalarial IgG rates were higher in the migrant population, although not markedly so, and say little regarding the source of their previous exposure, the interior areas versus the riverine areas.

TABLE 11

Age distribution of malaria slide positivity in the Ituxi River population by species of malaria.

	Number of Positive Slides	
Age Group	Plasmodium falciparum	Plasmodium vivax
0-4	15	20
5-9	10	15
10-14	12	6
15-19	4	1
20-24	0	1
25-29	1	2
30-34	1	2
41-49	0	0
$\geq$ 50	0	0
Totals	43	47

TABLE 12

Percent positivity of antimalarial serologies of the Ituxi River population by age.

	Percent Postive Serologies	
Age Group	IgG	IgM
0-4	81	0
5-9	67	4
10-14	87	18
15-19	92	21
20-24	100	36
25-29	100	53
30-40	97	47
41-49	100	33
$\geq$ 50	95	54

TABLE 13

Comparison of antimalarial seropositivity between a group of migrants to the jungle interior and a non-migrant riverine population, Ituxi River study area.

	Percent Serological Positives	
Population	IgG	IgM
Migrants	92.4	26.4
Non-migrants	86.8	37.7

In another attempt to clarify the transmission patterns of malaria within this area, a group of 42 persons who travel the river by boat, but who do not live in the study region, were studied serologically. Of these, a total of 27 (64.28%) were positive for IgG and 13 (30.95%) were positive for IgM. Since the rate of positive serologies in this group, particularily for IgM, does not differ markedly from that of the resident population, the impact that these river travers have on the overall transmission patterns of malaria is questionable. However, these persons may play an important role in the annual reintroduction of P. falciparum into the study area after the apparently temporary disapperance of this parasite during the dry season of certain years. This contrasts with P. vivax which occurs at reduced, but still significant, levels throughout the dry season, mainly in the form of recrudescent disease. The mobile populations also may be effective carriers of malaria between family units during the malaria transmission season.

Further analyses of presently available data are continuing and more field studies in the Ituxi region are being planned for FY80.

4. Immunological studies of malaria in Amazonas, Brazil:

a. Introduction: The program in malaria immunology was established at the University of Brasilia in the Center of Tropical Medicine and Nutrition in October, 1978. Suitable laboratory space was selected, necessary physical modifications were made, and equipment was installed during a start-up phase of approximately four and one-half months. Since that time, much progress has been made toward the

initial goal of providing full laboratory support for the clinical and epidemiological studies of malaria presently being conducted from this center. Two technicians have been fully trained in all of the techniques utilized in the routine operation of the laboratory and efficiently assist in all aspects of the ongoing research. The malaria serological studies and the necessary support activities of this operation are functioning at a level of proficiency to allow expanded efforts in the areas of drug susceptibility testing and the study of the in vitro cultivation characteristics of local strains of P. falciparum as they are obtained from the various study areas.

b. Objectives: The objectives of the malaria immunology program are to perform:

1) malaria serological testing;

2) drug susceptibility testing of Brazilian strains of P. falciparum.

3) collection, cryopreservation, and storage in stabilate form of strains of P. falciparum to provide material for ongoing studies and to serve as a reference in monitoring future patterns of drug susceptibility in the Amazon region of Brazil;

4) laboratory support of ongoing studies of patients with tropical splenomegaly syndrome from the Ituxi study region; and

5) other tests, such as the species-specific indirect fluorescent antibody test, to study the immunologic characteristics of malaria in Brazil as logistical capability permits.

c. Methods: Methods used to accomplish these objectives are:

1) maintenance of a constant and dependable source of malaria antigen by the on-site in vitro cultivation of P. falciparum (2,3) using blood components locally available from the teaching hospital in Sobradinho, DF, Brazil;

2) routine use of the indirect fluorescent antibody test (IFAT) as the standard serologic test for determining levels of antimalarial antibodies (4) using commercial anti-IgG and anti-IgM fluorecein-labelled globulins;

3) use of the in vitro technique of chloroquine susceptibility testing (5) to study the drug resistance patterns of local strains of P. falciparum.

4) development of the species-specific malaria IFAT (6) using locally obtained P. vivax antigen from patients infected with this organism as logistical capabilities permit; and

5) quantitative determination of IgM levels, particularly in patients with tropical splenomegaly syndrome, by the radial immunodiffusion assay system (7) using commercially-acquired kits.

d. Progress: In February, 1979, the in vitro cultivation of P. falciparum (Strain Cb1, Department of Immunology, Walter Reed Army Institute of Research) was initiated using the tissue culture flask/mixed gas system. This strain has demonstrated excellent growth characteristics and, until recently, has served as the standard laboratory strain for antigen production. The cultivation has been interrupted voluntarily on various occasions by cryopreservation of the stock material and then restarted by deglycerinization as the need for antigen to prepare slides for the IFAT has dictated. The candle jar culture system using either standard Petri dishes or 96-well microtiter plates has been incorporated with excellent technical results, and in many ways is preferred over the flask system because of its greater simplicity, economy, and the facility of medium changes.

On 25 July 1979, at a field site on the Ituxi River, one strain of P. falciparum from an untreated patient was cryopreserved. A total of 7 NUNC tubes of stabilate was prepared in the field and 2 of these were used to inoculate 4 flasks of medium 1640-HEPES/10% fresh frozen plasma and fresh, washed erythrocytes on 4 September 1979. (Unforeseen problems encountered in scheduling air transportation of the liquid nitrogen cannister back to Brasilia account for the delay between time of collection of this strain and subsequent cultivation attempts). Active parasite growth occurred in these initial cultures and the strain continues to thrive in continuous cultivation. It has presently been maintained for more than one month in this system. High parasitemias are obtained easily and two lots of antigen slides have already been produced, as well as additional organisms for cryopreservation. It has also been placed into the candle jar system and studies of its in vitro growth characteristics are in progress.

Initial observations indicate that this strain exhibits a rate of growth in culture similar to or slightly higher than strain Cb1. The new strain has been given the name "Ituxi 084", after the location in which it was collected and the computer card number of the patient. The cultivation of this strain provides a preliminary indication that many such strains may be adapted to the continuous culture system to allow in depth investigation of their drug susceptibility patterns. This strain is presently being studied in the in vitro chloroquine susceptibility test system and useful data will be available in the near future pertaining to its pattern of drug response.

The technical capability to perform in vitro drug susceptibility testing in the field presently exists. On the field trip earlier

in CY 1979, virtual cessation of P. falciparum transmission due to an unseasonably severe dry season precluded the large scale implementation of this test, however. Additional field trips later in CY 79 to a study area near Manaus and early in CY 1980 to Ituxi are planned with the expressed objectives of performing field drug testing and collecting additional strains for laboratory cultivation and study. These studies should provide much objective data regarding the current prevalence of chloroquine-resistant P. falciparum in several fairly representative areas of the Brazilian Amazon basin.

Since March, 1979, the IFAT has been in full, routine operation to support the ongoing epidemiological studies of malaria from this center. Depending on the number of designated readers, 48 or 96 tests have been performed on a daily basis since the initial standardization of this procedure. Excellent technical results are being obtained from the commercially-acquired antiglobulins and the P. falciparum antigen cultivated here in the laboratory.

In addition to the routine serological determinations, the preliminary results of which were presented above, a study was conducted to evaluate the applicability of the filter paper method of collecting blood for subsequent serological testing. The study material consisted of one group of sera and two groups of filter paper specimens obtained simultaneously from the same patients. The sera and one group of filter papers were stored at - 20°C from the time of collection to time of testing, while the second group of filter papers was stored at room temperature for this same period. Approximately two months elapsed between time of collection and subsequent testing in the IFAT system. The resultant data (Table 14) indicate that neither group of filter paper specimens compared favorably to the serum samples in demonstrating the presence of antimalarial antibody, either IgG or IgM. These data, in addition to similar findings obtained from other filter paper specimens from the Ituxi population, indicate that this method of sample collection is almost certainly producing many false negative results and will not be used on a routine basis in further serological studies of malaria on the Ituxi River.

Preliminary studies are proceeding with the radial immunodiffusion assay of IgM antibody levels in a small group of tropical splenomegaly patients from the Ituxi study area. Initial data (Table 15) indicate that high levels of circulating IgM are present in these patients. The titers of malarial antibodies, also presented in Table 15, confirm that at least a portion of this circulating IgM is specific for malaria, although as has been earlier observed in this syndrome (8), other types of IgM appear to occur in significant quantities in these patients. Further investigation of these relationships is proceeding.

TABLE 14

Malarial antibody positivity rates in a controlled study of serum and filter paper specimens simultaneously collected from the same individuals, Ituxi River study area.

Sample	Percent Positive	
	IgG	IgM
Serum (-20°C storage)	100	25.5
Filter papers (-20°C storage)	72.3	6.3
Filter papers (Ambient temperature storage)	27.6	0

TABLE 15

Antimalarial IgG and IgM titers by the immunofluorescent antibody test and total circulating IgM levels by radial immunodiffusion assay in four tropical splenomegaly patients, Ituxi River study population.

Patient	Malarial antibody titers		Level of total circulating IgM (mg/dl)
	IgG	IgM	
M. S. O.	1:1280	1:80	460
M. V. P.	1:320	1:80	665
F. S. O.	1:1280	1:320	2300
C. S. O.	1:5120	1:1280	2300

5. Entomological Studies on Malaria in Amazonas, Brazil.

a. Introduction: The Amazon Basin is classified as "refractory" to malaria control efforts. This classification is based on the persistance of malaria transmission in spite of the control program. The success of the program rests on controlling the vector populations by house spraying with DDT. Obviously, "refractoriness" indicates that due to some condition or complex of conditons the treatment of houses with DDT does not interdict malaria transmission as expected. As part of an integrated approach to define the causative factors for continued malaria transmission, emphasis in the vector studies has been to establish the broad parameters of vector behavior that have direct impact on the effectiveness of house spraying with DDT. Our field work along the Ituxi River brought us in contact with another entomological phenomenon that might influence the effectiveness of malaria vector control efforts. This occurs in the form of a male bee that seems to actively remove DDT from treated houses. Preliminary observations have been made on these bees; thus, the results of our entomological investigations will be presented in two separate categories as 1) the ecology and populations dynamics of malaria vectors and 2) the role of euglossine bees in the removal of DDT from sprayed houses.

b. The Ecology and Population Dynamics of Malaria Vectors.

1) Objective: To describe the behavioral, morphological and physiological characteristics of the malaria vectors in Brazil, with special emphasis on Anopheles darlingi Root.

2) Background:

The definitive research efforts on malaria in the Amazon region were conducted from 1930-1950 (9, 10, 11, 12, 13). Results from these studies revealed the principal vector in the Amazon interior to be Anopheles darlingi Root. Secondary vectors were found to be An. (Nyssorhynchus) albitarsis Lynch Arribalzaga and perhaps An. (Ny.) brasiliensis (Chagas). The major vector is generally considered to be a riverine mosquito and most studies in the epidemiology of malaria have been conducted in riverine semi-urban hatitats.

Anopheles darlingi is the most important vector of malaria in South America (14, 15). Because this species prefers sunlight, its greatest density is along major river valleys, and it proliferates wherever human activities result in the removal of shade-producing forest. An. darlingi is also strongly attracted to man and rests indoors (14, 16). It seems that An. darlingi are still physiologically susceptible to DDT and only recently has behavioral resistance to

DDT been reported (17). Unfortunately, no supportive data have been presented to quantify that observation.

In forest areas away from rivers, other species may transmit malaria secondarily (18). These species are shade tolerant and commonly bite and rest outdoors (14). Control by the application of residual insecticides to the interior walls of dwellings is therefore of limited value. Species which have been incriminated as secondary vectors in northeastern South America include Anopheles nuneztovari, An. triannulatus, An. oswaldoi, An. brasiliensis, An. mediopunctatus, An. albitarsis, An. bellator, An. cruzi, and An. homunculus (14, 16). However, the exact role of secondary vectors in the maintenance of malaria has not been clarified.

Anopheles nuneztovari is probably a complex comprising at least two species in northern Brazil, with some of the species being malaria vectors, others not (19, 20). There are undoubtedly other groups of sibling species among anopheline species in Brazil that are malaria vectors, e.g., An. oswaldoi. In many instances, resolution of the morphological forms and geographical strains of anophelines transmitting malaria can be accomplished only after the study of a series of individually reared specimens from many geographic areas, and in some cases, only after the comparative study of chromosome morphology (15).

3) Methods:

An entomological survey of the peridomiciliary environments along the Ituxi River was conducted in July and August 1979 (Fig. 1). The survey consisted of conducting human bait collection near or in houses at sunset and of opportunistically dipping for larvae in various types of water. In the latter part of this trip a sequence of 3 all night biting collections were conducted at Floresta (Fig. 1). Collections were conducted for 30 min each hour with one team of collectors (2 men per team) in a house open on three sides while another team collected in an open area about 20 m from the house. Each hour the teams were rotated between sites and the third night the team members were changed. In addition, 2 tests for physiological resistance of An. darlingi to DDT were conducted. Test specimens were wild caught females from human bait collections. Prior to setting up each test the females were observed for 3-4 hours to identify and remove any damaged specimens. Females were not given sugar water; but were furnished with pads soaked in plain water following a 1 hr exposure to DDT treated papers. The world Health Organization test kit and test procedures were employed to conduct these tests (21).

Results from the above studies emphasized the need for an experimental house for more detailed studies on the behavior of darlingi populations. House construction at Floresta was initiated in October 1978 and completed in January 1979. The house was constructed with 1) a palm thatch roof, 2) walls constructed of palm slats, and 3) one small room with a wood plank floor and another of palm slats. The wood plank floor provided the necessary stability for work with a microscope, etc. All windows (8 in total) were of equal size so entrance and exit traps would be interchangeable. The house was wired for electricity provided by a 3 KVA generator.

The first series of detailed studies was conducted in February - March 1979, and follow-up observations were made in May - June 1979. The following study methods were employed:

a) Paired, outdoor-indoor human bait collections were conducted in a uniform manner throughout the night and day to determine the indoor-outdoor patterns of biting activity. Collections were conducted 15 min/hr by one person at each site. Collectors were continually rotated between collecting sites and teams were switched every 6 hours. Furthermore, teams were rotated between shifts every night. The all night collections were conducted simultaneously with the entrance-exit trap collections. Two series of collections throughout the day were conducted. Data will be reported from series conducted 24 - 27 February 1979 and 31 May - 7 June 1979. Temperature and humididy, was recorded every 6 hours for the first series and at hourly intervals for the May-June investigations.

b) Entrance and exit traps placed in windows were collected at 2 hr intervals throughout the night (1800-2000, 2000-2200, etc). Each trap had a sleeved opening for removing captured specimens with a mechanical aspirator. A Safari Fluorescent lamp was used to illuminate the trap interior after the entry portal to the trap was closed with towels. We began closing the traps after we observed blood engorged ♀♀ darlingi actively entering traps in response to the bright fluorescent light, i.e., they demonstrated a positive phototaxis. All specimens were identified and individuals from exit traps were examined for age grading of blood meals according to Sella's scheme for stages of blood meal digestion and ovarian development (22).

c) Sella's method for evaluating the stages of blood meal digestion and ovarian development was employed with specimens caught in exit traps to determine elapsed time after engorgement. A study was conducted in February 1979 to evaluate Sella's criteria with An. darlingi at in-house temperatures. This study was conducted by holding individual engorged females for variable periods of time; they were then killed and inspected for concordance with one of the

stages proposed by Sella. A total of 100 females, engorged on human blood, were included in this study.

d) Blood-engorged An. darlingi were collected in the peridomiciliary environment during the early evening, marked with USR fluorescent pigment 1953 and released in the house at 2200. Marking was accomplished by blowing the powder into a small holding cage containing the specimens. Periodic observations with a Black-Ray, ULV.56, long wave ultra-violet lamp were made following release to determine the preferred resting site of blood-fed specimens. These studies were conducted on 2 separate occasions (22 and 28 February 1979) and 100 specimens were marked for each study.

e) Two tests were conducted to determine the preferred resting sites of unfed specimens and the time of feeding during the night. Specimens were collected from the entrance traps, during the 1800-2000 hour interval, marked and then released within the house at 2040 hr. All specimens caught in the subsequent hourly human bait collections were inspected for the presence of marked specimens.

f) A series of resting collections were conducted 28 February-1 March 1979. Three separate collections were conducted for 5 minutes simultaneously inside the house, from the external walls and from vegetation near the house. Two series were conducted in the evening at half hour intervals from 1830 to 2105 hr. and one series in the early morning from 0540-0715. Resting adults were captured with a mechanical aspirator.

g) Studies were undertaken on the distribution of host-seeking An. darlingi populations by distance from the peridomiciliary environments. A single collector was stationed at each of 3 sites: one > 10 m from the house, another at 20 m and the 3rd at 40 m from the house. Collections were conducted simultaneously for 15 min each from 1750 to 2005 on 3 and 4 June 1979.

4) Progress:

Anopheles darlingi were consistently present in the peridomiciliary habitats along the Ituxi and Uaquire River systems. This species was also found along the lower reaches of the Endimari River. The entire River network is sparsely populated with single family dwellings that commonly have associated populations of An. darlingi.

Data from the insecticide resistance tests are presented in Table 16. The LC_{50}, estimated on log probit graph paper, with combined data from 2 tests, is 0.72% DDT and clearly within the susceptible range.

TABLE 16

Result from 2 tests for physiological resistance to DDT of ♀♀ Anopheles darlingi Root. Females were caught in human bait collections from 1830-2030 hr at Floresta, Ituxi River, Amazonas, Brazil. Tests were initiated at 2300 hr on the night of collecting the test specimens.

Test No.	% DDT	Number tested	Number moribund or dead
1*	0.0	40	5
	0.5	40	17
	1.0	36	28
	2.0	39	36
	4.0	38	38
2**	0.0	30	4
	0.5	39	22
	1.0	24	12
	2.0	42	39
	4.0	41	41

* Test performed 13-14 July 1978.

** Test conducted 1-2 August 1978.

A bimodal pattern of biting activity was documented for An. darlingi both inside and outside of a non-enclosed house at Floresta in August 1978. Peak activity was during, and immediately after, sunset and at sunrise (Fig. 2). It is significant that no marked differences were found in the activity cycles of darlingi in the house and in an open area near the house.

Two separate series of studies on activity patterns in a house with complete walls were conducted. Uniform methods were applied during both; thus, findings are presented as combined results with reference to the separate series as study 1 (February - March 1979) and study 2 (May - June 1979). Weather conditions were different for studies 1 and 2 with temperature range limits of 24-31°C recorded for study 1 and 16-30°C for study 2.

A bimodal pattern of biting activity in the peridomiciliary environment (within 10 m of the house) was documented in the August 1978 series of human bait collections and in studies 1 and 2 in 1979 (Figs. 2, 3 and 4). Peak activity occurred during, and preceeding, sunset with a secondary peak at sunrise (at approximately 0600). The secondary peak was not well expressed in the study 2 collections. These activity patterns were compared by calculating cumulative per cent distributions for each and testing in the Kolmogorov-Smirnov two sample test (23). No significant differences were detected in these analyses.

Human bait collections were conducted inside the experimental house, during studies 1 and 2, to determine the pattern of activity within a completely enclosed house. Collections in study 1 revealed a sharp increase in activity after sunset with more or less continuous activity throughout the night. There was no detectable peak in activity at sunset or sunrise. The minimum temperature recorded during these collections was 24°C.

In-house biting activity during study 2 was most intense at, and 3 h following, sunset. After 2147 the activity dropped and remained low the rest of the night. A comparison of results from studies 1 and 2 with the Kolmogorov-Smirnov two sample test revealed significant difference ($p < 0.01$) between the 2 activity patterns.

It is reasonable to explain deviations form expected activity patterns by notable differences in study conditions. Therefore, we hypothesized that low temperatures suppressed host-seeking activity of An. darlingi during study 2. We tested this hypothesis by anlyzing sequential collections for 2 activity intervals with the Kendall Rank Correlations and Kendall Partial Rank Correlation Coefficients (23). Data available for analysis consisted of numbers collected per collection, time of collection and temperature at the time of

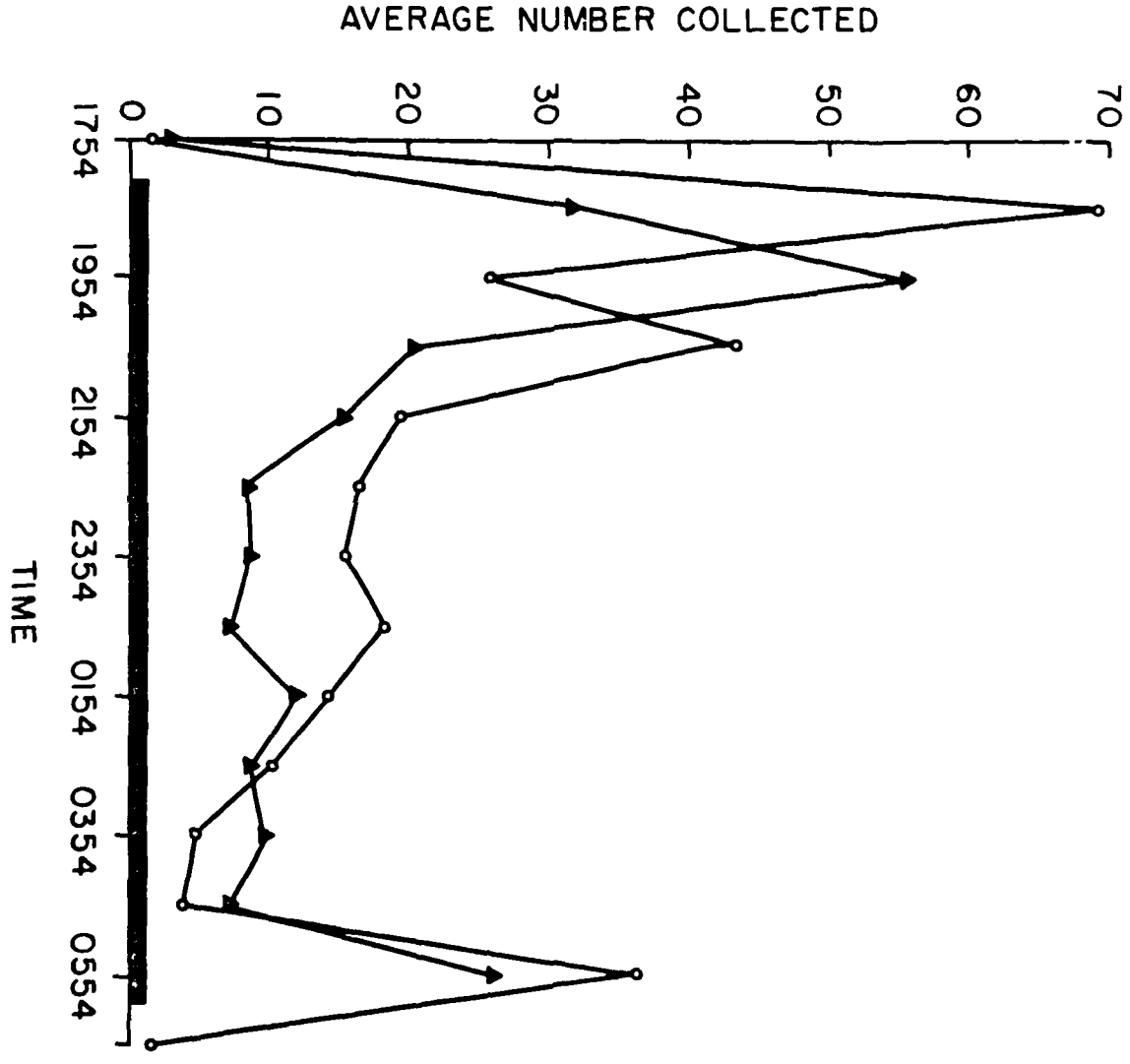


Figure 2. Numbers of Anopheles darlingi Root from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil in August 1978. Collections conducted by 2 collectors for 30 min. each hour (o——o inside of a house with one wall only; o——▲ open area near the house).

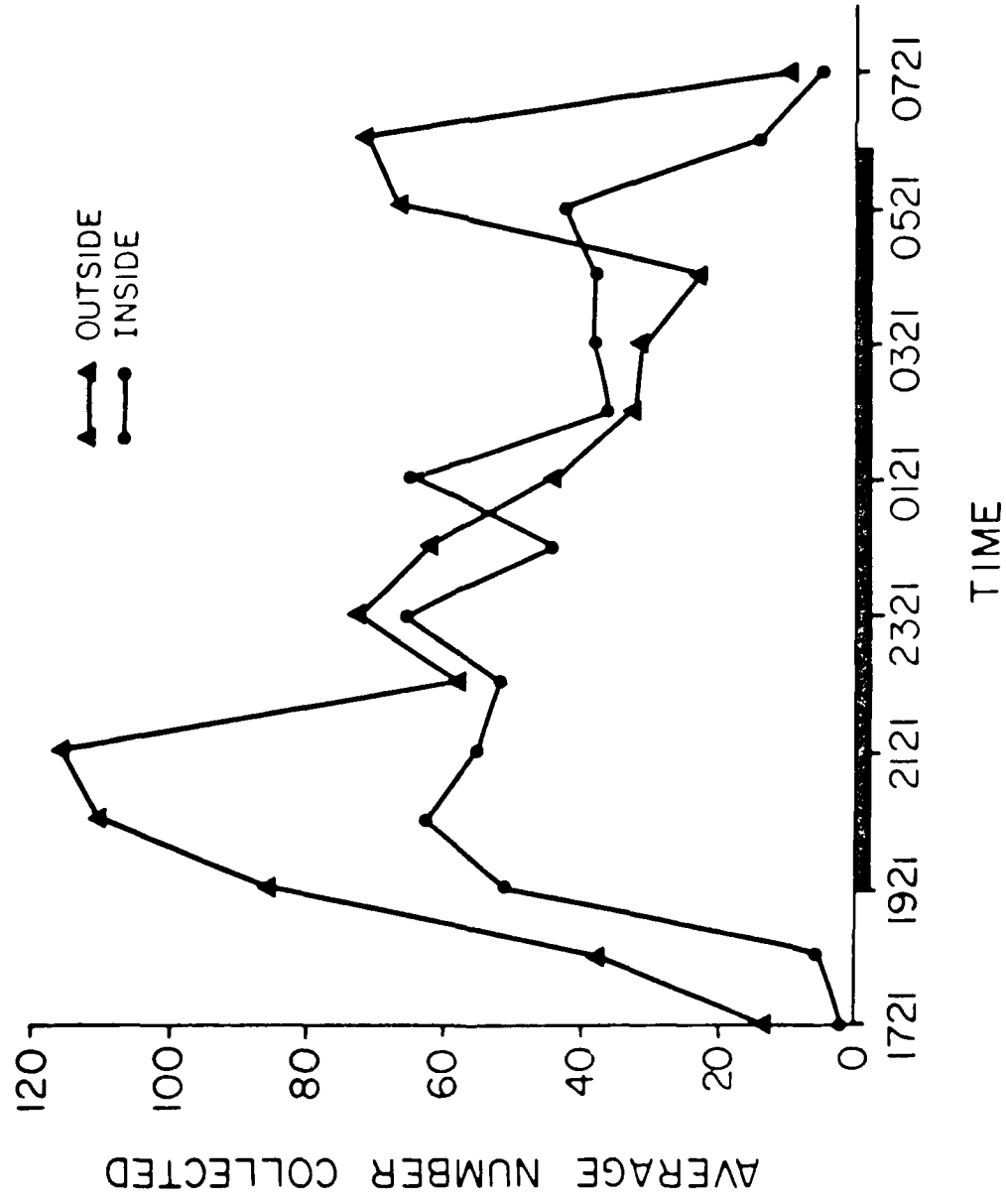


Figure 3. Numbers of Anopheles darlingi Root from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil in February 1979. Collections conducted by 1 collector each in the house and within 10 m of the house for 15 min. each hour (plotted by midpoint of collection intervals).

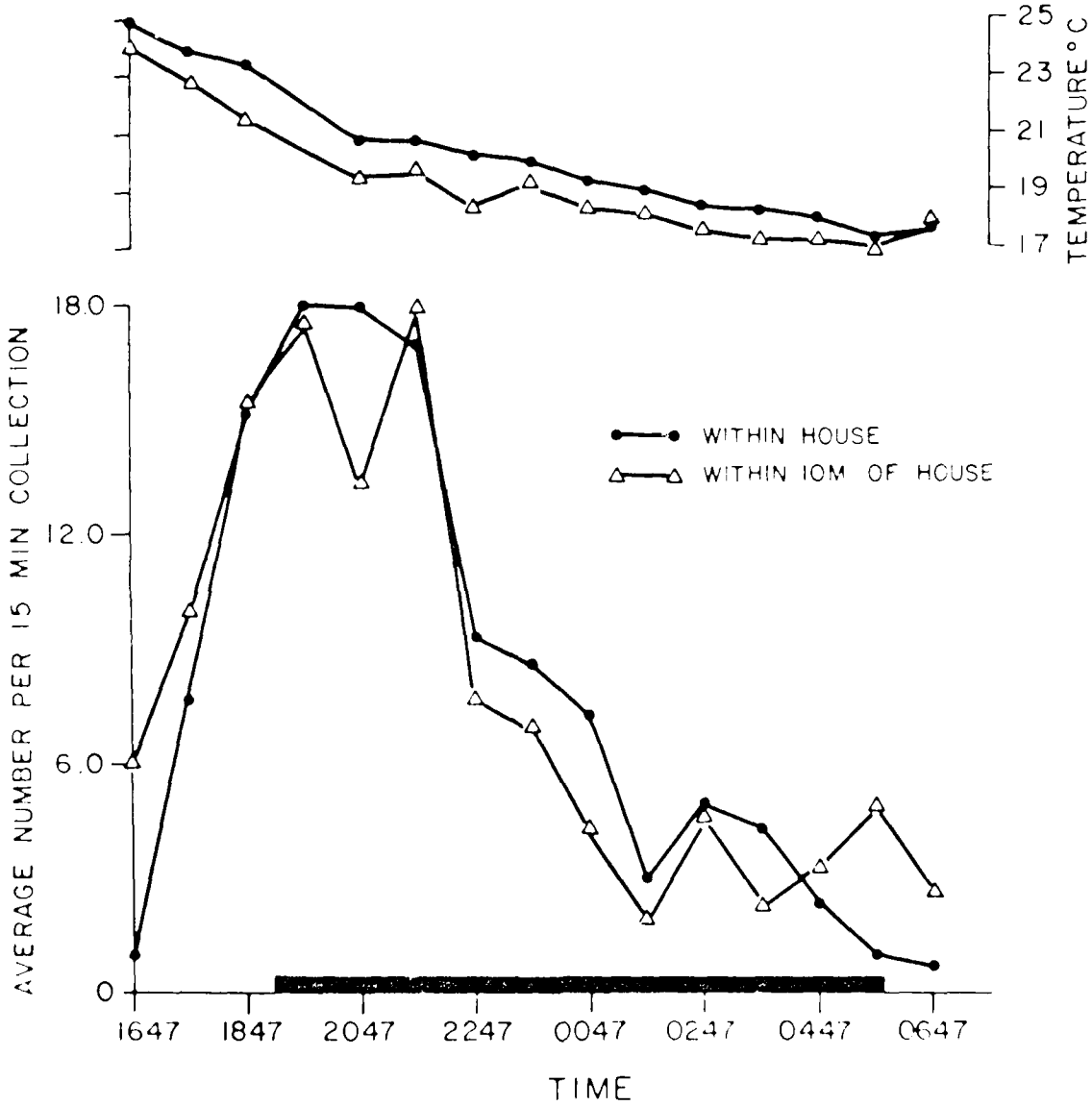


Figure 4. Numbers of Anopheles darlingi Root from 3 nights of human bait collections at Floresta, Ituxi River, Amazonas, Brazil, 31 May-3 June 79. Collections conducted by 1 collector each in the house and within 10 m of the house for 15 min. each hour (plotted by midpoint of collection intervals).

each collection. Objectives of this test procedure were to determine the relative contributions of collection time and temperature to numbers collected. The problem of endogenous activity rhythm influence on numbers collected by chronological time was minimized by testing 3 nights of sequential data from defined activity intervals. Our cumulative data revealed 4 activity intervals for An. darlingi in the peridomiciliary environment, viz., very low activity during the day, peak activity for about 3 hr during and after sunset, moderate to low activity during the 2200 - 0530 interval and a secondary peak of intense activity for < 30 min. at sunrise (0600 ± 10 min). Therefore, separate tests were conducted on all collections from the 2 intervals, 1835 - 2055 and 2345 to 0500. Admittedly, the in-house activity patterns from study 1 did not reveal the 4 activity intervals as described. However, we believe data from outside biting collections reflect actual endogenuous rhythms and that the process of seeking and gaining entry into the house from 1800 - 2200 hrs (Fig. 6) is another expression of peak activity at sunset. It seems likely that the continuous biting activity within the house results from the population responding to a different set of feeding "cues" than populations feeding outside the house.

The mechanics of the test procedure consisted of calculating separate Kendall Rank Correlation Coefficients for numbers collected *vs* temperature, numbers collected *vs* time and temperature *vs* time for data from both activity intervals. Tests of significance were performed on the r values at the 0.01 level of probability. The r values were then employed in the Kendall Partial Rank Correlation Coefficient to parcel out the time and time-temperature effects. No tests of significance are available for the resultant $r_{xy.z}$ values. Results of data analysis from both activity intervals are presented below. High roe values for numbers collected with different temperatures indicate that temperature was the main determinant for number collected within the activity intervals.

Systematic collections outside the house were initiated during study 2, 3-7 June 1979, to characterize the level of biting activity during the day and to document the crepuscular peaks in biting activity with warmer ambient temperatures. The frequency of human bait collections were increased during the early morning and evening to more precisely document periods of peak activity. We verified the previously reported observation that the morning peak is intense and of short duration (Fig. 5). Also the early evening peak was duplicated in these series of collections. Data from collections conducted throughout the day demonstrated the absence of biting activity only in the early afternoon.

Results from entrance and exit trapping of darlingi during study 1 and 2 demonstrated a surge of numbers entering the house

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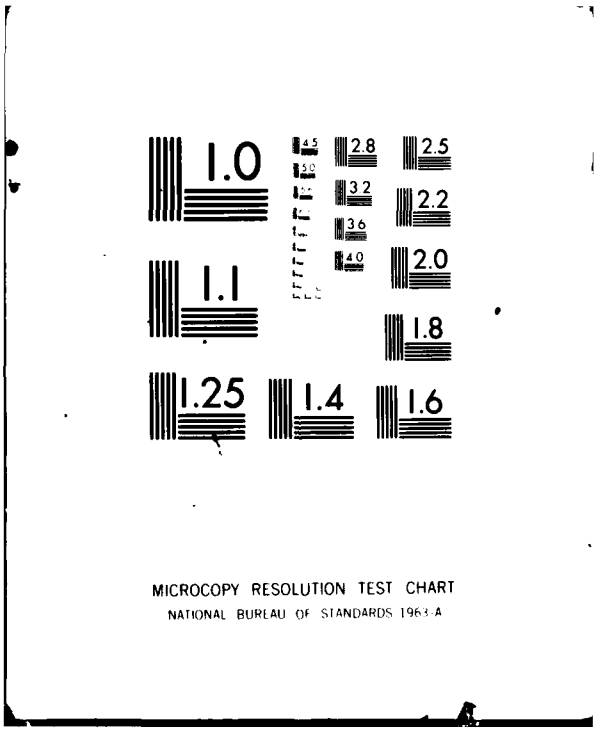


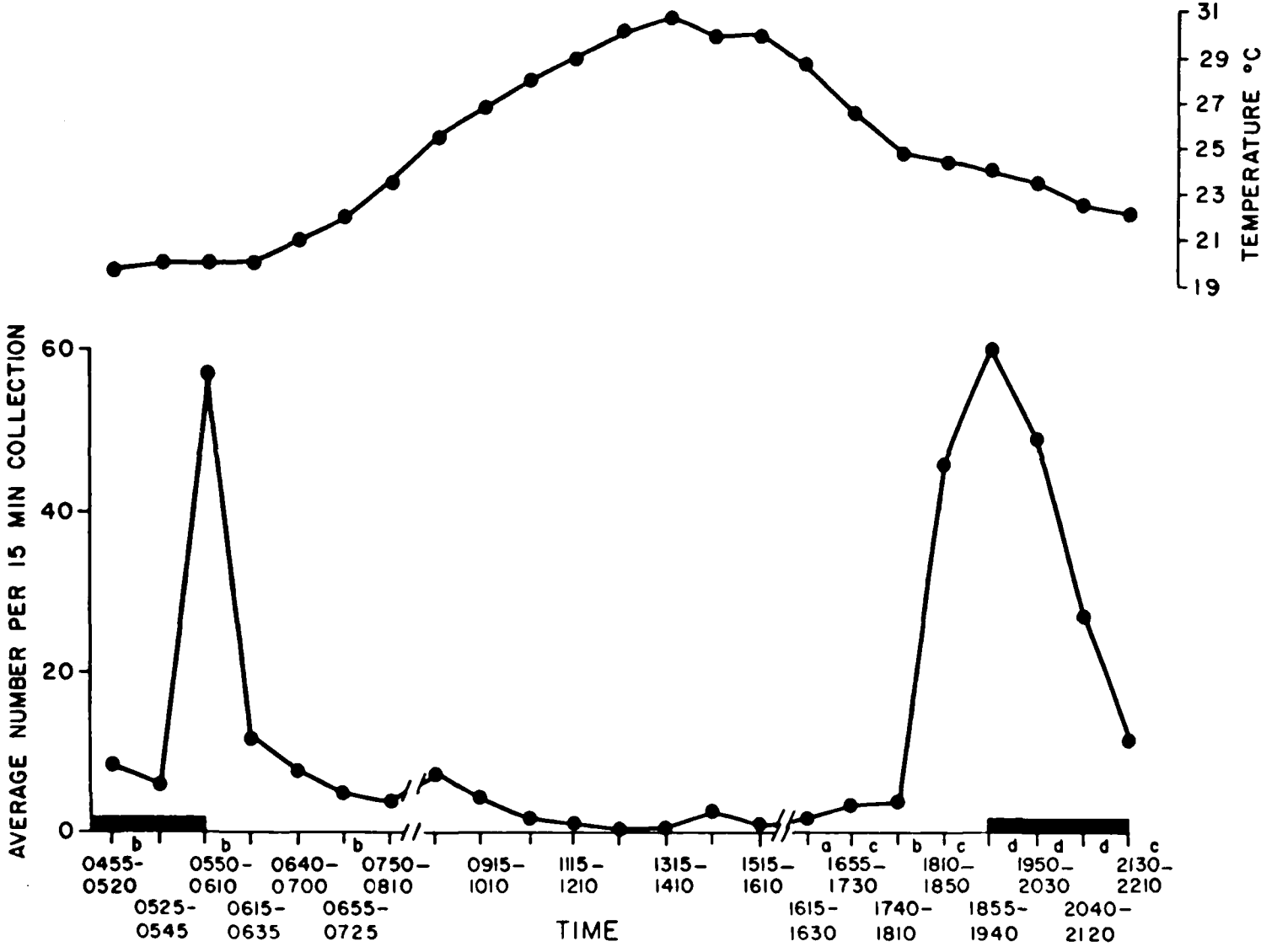
Figure 5. Numbers of Anopheles darlingi Root from 2 days of human bait collections at Floresta, Ituxi River, Amazonas, Brazil in June 1979. All collections were conducted by 1 collector within 10 m of the house for 15 min each (plotted by time intervals).

[a] Value based on a single 15 min collection.

[b] Average from 3 collections.

[c] Average from 4 collections.

[d] Average from 5 collections.


31
29
27
25
23
21
19
TEMPERATURE °C
60
40
20
0
AVERAGE NUMBER PER 15 MIN COLLECTION
b
b
b
a
c
b
c
d
d
d
c
0455-
0520
0525-
0545
0550-
0610
0615-
0635
0640-
0700
0655-
0725
0750-
0810
0915-
1010
1115-
1210
1315-
1410
1515-
1610
1615-
1630
1655-
1730
1740-
1810
1810-
1850
1855-
1940
1950-
2030
2040-
2120
2130-
2210
TIME

Analysis of data from two activity intervals.

Activity Interval (time)	Variables	r	$r_{xy.z}$
1835-2055	r_{xy} = Number Collected *vs.* Temperature	0.70**	———
	r_{xz} = Number Collected *vs.* Time	0.04	———
	r_{zy} = Temperature *vs.* Time	0.01	———
	Kendall Partial Rank Correlation Coefficient =		0.70
2345-0500	r_{xy} = Number Collected *vs.* Temperature	0.64**	
	r_{xz} = Number Collected *vs.* Time	0.41	
	r_{zy} = Temperature *vs.* Time	0.53*	
	Kendall Partial Rank Correlation Coefficient =		0.55

* Significant at 0.01 level of probability ($p < 0.0027$).

** Significant at 0.01 level of probability ($p < 0.0007$).

1800-2200 hours (Figs. 6 and 7). Exodus from the house did not begin until 0400 hours. Again, there were marked differences in the study 1 and study 2 collections results. The entrance of females peaked earlier (1800-2000) and was of short duration in study 2; in addition, movement out of the house started later (0600-0800) and continued through mid- to late-morning. These differences are perhaps another expression of the temperature influence on the activity of darlingi populations.

Based on data presented in Figures 6 and 7 it seems that darlingi enter the house in the evening, with peak activity between 1800 and 2200, remain in the house until sunrise and exit. Data from both studies indicate that very few specimens remain inside the house during the day and rarely did gravid females appear in the exit traps. The preponderance of late fed specimens in exit traps at

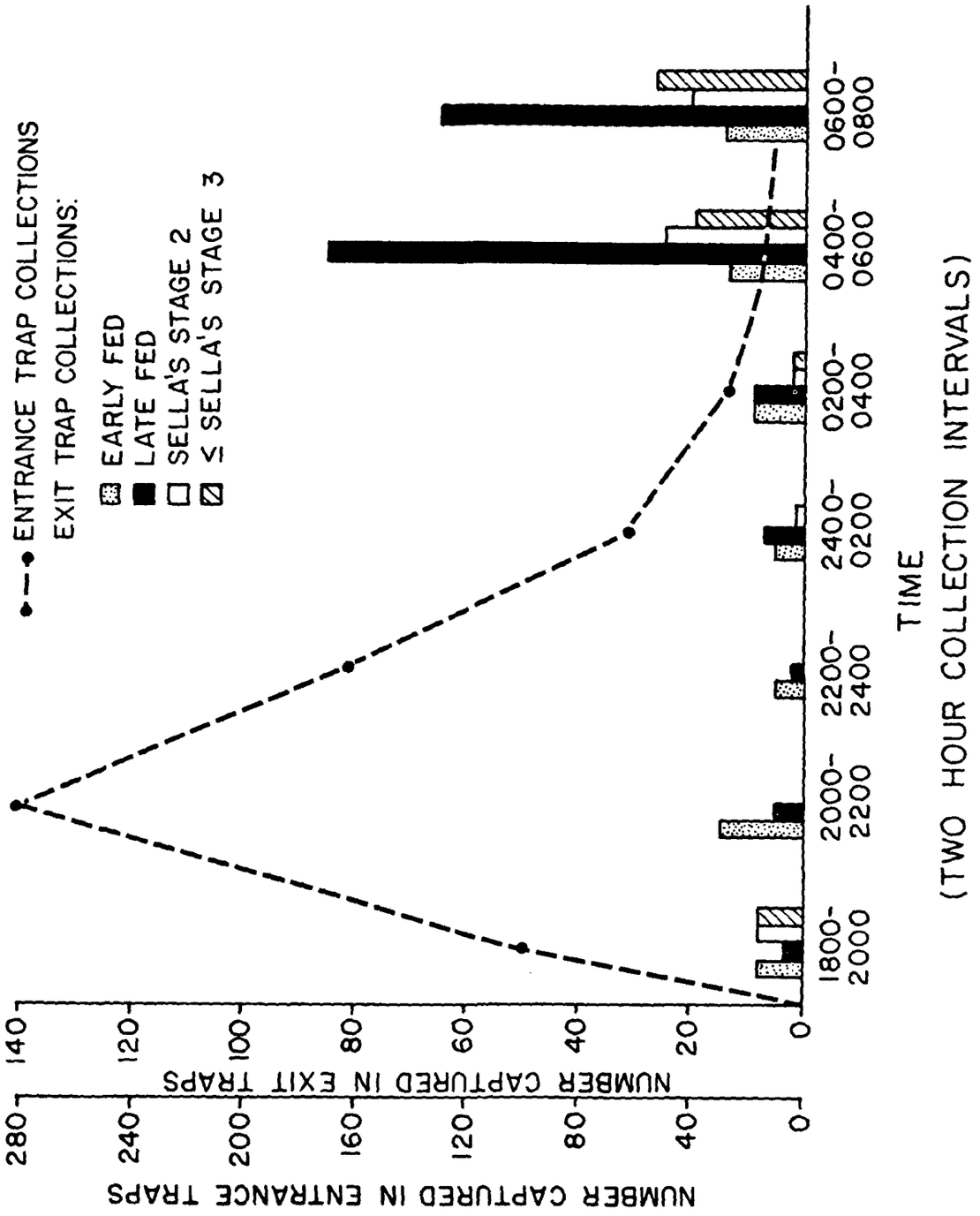


Figure 6. Numbers of Anopheles darlingi Root from trapping with 3 entrance and 3 exit traps at Floresta, Ituxi River, Amazonas, Brazil in February 1979.

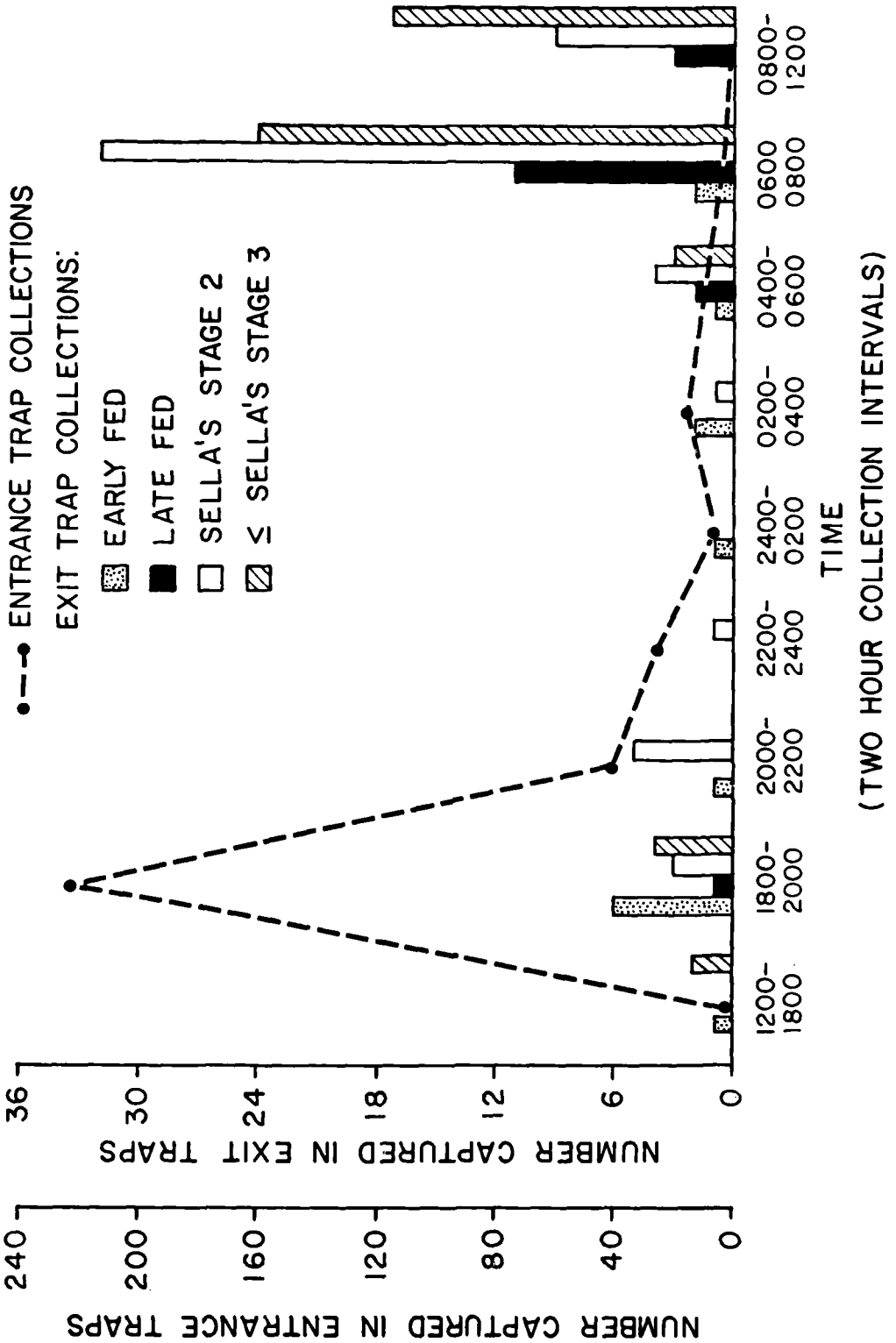


Figure 7. Numbers of Anopheles darlingi Root from trapping with 3 entrance and 3 exit traps at Floresta, Ituxi River, Amazonas, Brazil, 31 May - 3 June 1979.

sunrise in study 1 is compatible with a continuous pattern of biting activity throughout the night (Fig. 3). Whereas, the greater number of Sella 2 specimens, recorded in exit traps during study 2, results from the early evening peak in biting activity (Fig. 4).

Observations on the rate of blood digestion and ovarian development in 100 female An. darlingi were conducted during the study in February, 1979. The study specimens were maintained at ambient temperatures in the experimental house. All early fed females were clumped in the 1-5 hr. interval (Table 17). Specimens classified as late fed were found in both the 1-5 and 6-10 hr intervals; Sella 2 individuals were clumped in the 6-10 hr intervals and the majority (79%) of Sella 3 specimens were in the 11-20 hr interval. Since more Sella 3 specimens were in 6-10 hr interval than in 21-30 hr interval, it seems likely that most specimens attain this stage during the first part of the 11-20 hr interval.

Engorged An. darlingi, marked with fluorescent powder, were released inside the experimental house on 2 separate occasions during study 1 to determine their preferred resting sites. Periodic searches for marked specimens were made throughout the night. Although total numbers progressively declined with time after release, the majority of engorged darlingi were consistently found resting on the ceiling (Table 18). A search made outside the house prior to sunrise, revealed no particular preference for resting sites.

When unengorged specimens from exit trap collections were marked and released during study number 2, we again observed a preference for darlingi to rest on the ceiling (Table 19). This preference was particularily marked during the first 1.5 hours after release. There was a more equal distribution of numbers resting on the walls and ceiling later in the night. Again, we do not know if low temperatures recorded during study 2 influenced their selection of resting sites.

In addition to making observations on resting sites of marked, unengorged specimens released during study 2, we also tabulated numbers of marked specimens collected in exit traps (Table 20) and numbers collected in the hourly 15 min. human bait collections (Table 19 and Fig. 8). More than 50% of marked individuals collected from human bait were obtained during the first 3 hr after release (Fig. 8). In contrast, only 2 of 11

TABLE 17

Observations on the rate of blood digestion and ovarian development in ♀ ♀ *Anopheles darlingi* Root, at in-door temperatures and humidities*. Observations made with wild caught females on the Ituxi River, Amazonas, Brazil in February-March 1979.

Hours post-engorgement	Early fed	Late fed	Sella Stage 2	3	4	5	6	7	Undeterminded
1 - 5	14	5							0
6 - 10		7	6	4					3
11 - 20				19					1
21 - 30				1	6				2
21 - 40					1	7	5		1
41 - 50						4		5	0
51 - 60								9	0
TOTALS	14	12	6	24	7	11	5	14	7

*
Temperature range limits were 24° - 3°C.
Relative humidity range limits were 80 - 100%.

TABLE 18

Numbers of marked* ♀ ♀ Anopheles darlingi Root observed by resting site at intervals throughout the night in an experimental house at Floresta on the Ituxi River, Amazonas, Brazil. Two tests were conducted (22-23 February and 28 Feb - 1 March 79) and 100 specimens were collected in human bait captures, permitted to engorge, marked and released for each study.

Hours after release	Numbers seen						
	Inside			Outside			
	Floor	Wall	Ceiling	Wall	Under floor	Under roof overhang	Vegetation
0.25[b]	2	28	35	-	-	-	-
0.5[a]	8	22	27	-	-	-	-
1.0[b]	0	19	25	-	-	-	-
1.5[a]	0	9	29	-	-	-	-
7.25[b]	0	5	15	-	-	-	-
7.5[a]	0	3	5	2	3	0	2

*

Females were marked with USR pigment 1953 and marked specimens were subsequently identified with a Blak-Ray, ULV;56, long wave ultra-violet lamp.

a

Observations were made on 100 specimens released 22 February, 1979.

b

Observations were made on 100 specimens released 28 February, 1979.

TABLE 19

Number of marked* ♀ ♀ *Anopheles darlingi* Root observed by resting site at intervals throughout the night in an experimental house located at Floresta on the Ituxi River, Amazonas, Brazil. A total of 81 and 82 marked, unfed specimens were released 1 and 2 June 1979, respectively.

Hours after release	Number resting by site within the house				
	Floor	Walls	Rafters	Ceiling	Totals
0.5[a]	5	14	7	28	54
1.0[b]	0	10	4	27	41
1.5[a]	0	10	3	25	38
$\bar{X}$	1.7	11.3	4.7	26.7	44.3
4.5[a]	0	14	0	13	27
5.0[b]	0	10	1	7	18
$\bar{X}$	0	12	0.5	10	22.5
8.5[a]	0	7	0	11	18
8.5[b]	0	7	0	6	13
$\bar{X}$	0	7	0	8.5	15.5

* Females were marked with USR pigment 1953 and marked specimens were identified with a Blak-Ray, ULV;56, long wave ultra-violet lamp.

a Observations made on the 81 specimens released 1 June 79.

b Observations made on the 82 specimens released 2 June 79.

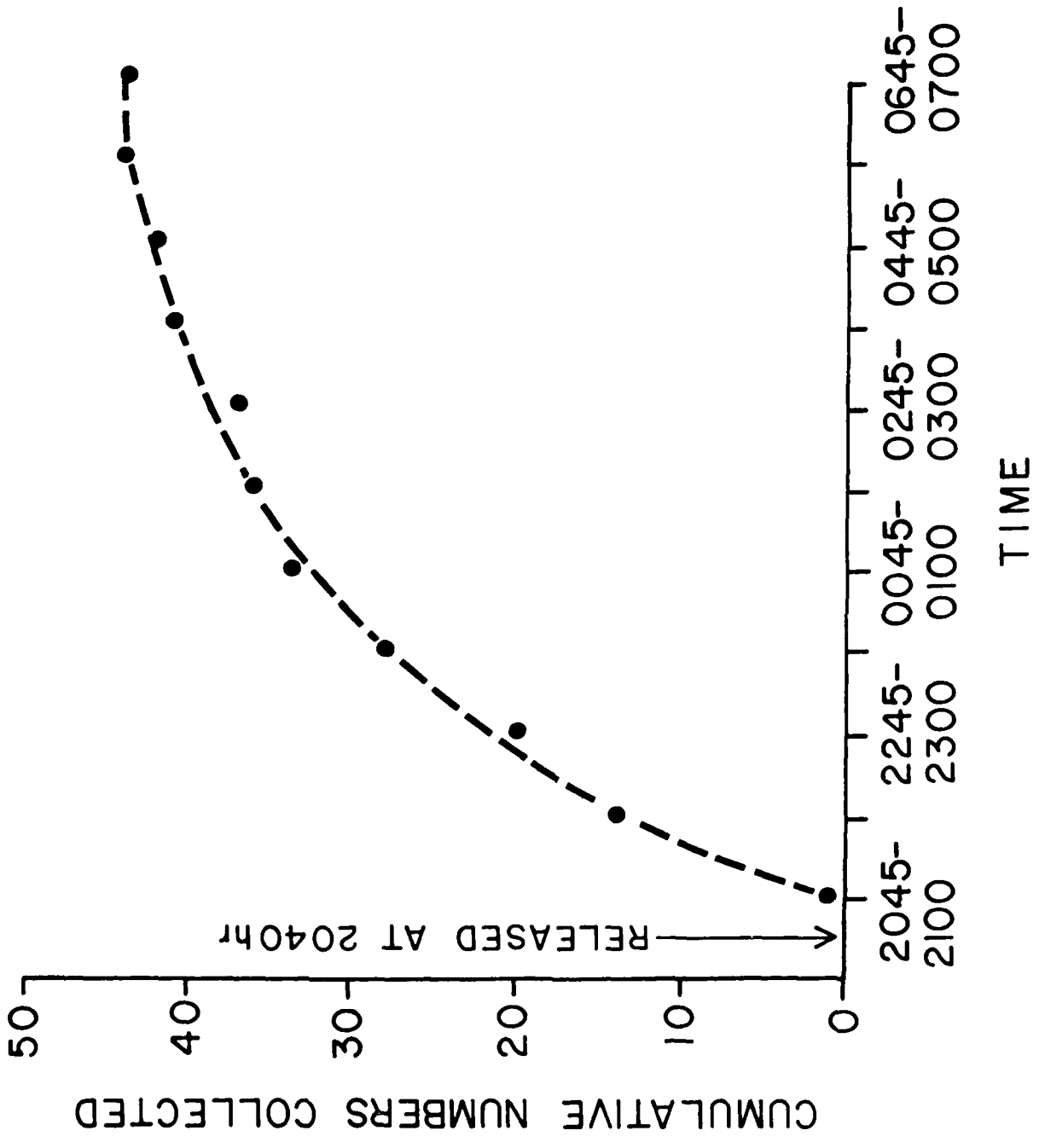


Figure 8. Cumulative numbers of marked Anopheles darlingi Root collected in human bait collections followitn release inside a house at Floresta, Ituxi River, Brazil, at 2040 hr. A total of 81 and 82 marked, unfed specimens were released 1 and 2 June 1979, respectively.

marked specimens that were captured in the exit traps were collected before 0600 hours (Table 20). After chronological adjustments were made in numbers of marked specimens, following removal by trapping (Table 21), we found a cumulative 32.9% of marked specimens were captured in human bait collections and 9.5% in exit trap collections.

The mark-recapture data, obtained during this study, is interpreted cautiously for the following reasons: a) very small numbers were recorded from the exit trap collections; b) marked specimens were released late in the period of peak biting activity (2045 hr); c) abnormally low ambient temperatures were recorded during both study nights; and d) the marked populations were discrete and do not reflect the variable but continual immigration and emigration of darlingi within the house.

The impact of these factors can be seen in the values of marginal totals in Table 20. The last column reveals that immediately following release, the marked specimens are more abundant in the exit trap collections than unmarked individuals, whereas engorged females are more abundant in subsequent collections. In addition, there were disproportionately few marked early to late fed specimens, a disproportionately large representation of unfed specimens (Sella 1), a proportionate number of Sella 2 and 3 specimens and no marked specimens in stages > Sella 3 (see marginal totals in last row of Table 20). This reflects the discrete characteristic of the marked population in that some exit immediately, none are in the house a sufficient time to be > Sella 3 and most feeding took place immediately, thus marked individuals were in Sella 2 and 3 at sunrise.

Calculations with the simple Lincoln Index (24) were performed to estimate total numbers of An. darlingi in the house with human bait and exit trap collection data. For these calculations, a = total number marked and released, n = number collected (marked + unmarked) after release, y = number recaptured after release and p = total number of darlingi in the house. The p value is calculated with the Lincoln Index formula:

$$p = \frac{an}{r} .$$

The a value was determined by multiplying the accumulative % collected by the number of marked specimens released (Table 2), e.g., for human bait collections it is 0.329 X 163 = 53.6 and for exit trap collections it is 0.095 X 163 = 15.5.

Calculations with the human bait collections gave a total of 504.8 An. darlingi in the house for the 2 nights (n = 166. a = 163 and r = 53.6). The estimate of populations size was 557 darlingi with

TABLE 20

Number of marked* ♀♀ *Anopheles darlingi* Root recaptured in 3 exit traps following release inside and experimental house on the Ituxi River at Floresta, Amazonas, Brazil. Combined data from releases of 81 and 82 marked, unfed specimens at 2040 hr on 1 and 2 June 1979, respectively.

Time	Early fed	Late fed	Sella stage 1	2	3	4	5	6	7	Total number captured (marked/unmarked)
2000-2200	0/0	0/0	2/0	0/1	0/0	0/0	0/0	0/0	0/0	2/1
2200-2400	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/0	0/0	0/1
0000-2000	0/1	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/1
0200-0400	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
0400-0600	0/0	0/0	0/0	0/2	0/1	0/0	0/0	0/0	0/0	0/3
0600-0800	0/0	1/6	2/4	1/3	2/6	0/2	0/0	0/0	0/1	6/22
0800-1200	0/0	0/1	0/0	2/2	1/2	0/0	0/0	0/0	0/0	3/5
Total number captured (marked/unmarked)	0/1	1/7	4/4	3/9	3/9	0/2	0/0	0/0	0/1	11/33

*

Females were marked with USR pigment 1953 and marked specimens were identified with Black-Ray, ULV; 56, long wave ultra-violtet lamp.

TABLE 21

Number available, number collected, % collected and accumulative % collected of marked* ♀♀ Anopheles darlingi Root in human bait and exit trap collections in an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Combined data from releases of 81 and 82 marked, unfed specimens at 2040 hr on 1 and 2 June 1979, respectively.

Time	Human bait collections				Exit trap collections			
	Number available	Number collected	% collected	Accumulative %	Number available	Number collected	% collected	Accumulative %
2000-2200	163	16	9.8	9.8	147	2	0.14	1.4
2200-2400	145	15	10.3	20.1	130	0	0	1.4
2400-0200	130	8	6.2	26.3	122	0	0	1.4
0200-0400	122	5	4.1	30.4	117	0	0	1.4
0400-0600	117	3	2.6	32.9	114	0	0	1.4
0600-0800	113	0	0	32.9	113	6	5.3	6.7
0800-1200	106	-	-	-	107	3	2.8	9.5
1200-1800	103	-	-	-	103	0	0	9.5

*

Females were marked with USR pigment 1953 and marked specimens were identified with Black-Ray, ULV;56, long wave ultra-violet lamp.

TABLE 22

Number of Anopheles darlingi Root in human bait collections conducted at 3 sites near an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Collections were conducted for 15 min. each at all 3 sites simultaneously, 3-4 June 1979.

Time	10 M from house			20 M from house*			40 M from house*		
	3June79	4June79	$\bar{X}$	3June79	4June79	$\bar{X}$	3June79	4June79	$\bar{X}$
1750-1805	6	5	5.5	2	7	4.5	10	10	10
1835-1850	40	33	36.5	12	6	9.0	0	10	5
1900-1915	127	58	92.5	4	15	9.5	10	6	8
1925-1940	44	21	47.5	1	23	12.0	15	2	8.5
1950-2005	87	20	53.5	2	21	11.5	0	7	3.5

*
Collection site located in a low secondary forest.

TABLE 23

Species and numbers of specimens captured in human bait collections conducted simultaneously at 3 sites (10 collections/site) near an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Collections were conducted for 15 min. each from 1740-2005, 3 and 4 June 1979.

Distance from experimental house		
10 meters	20 meters	40 meters
Anopheles (Nyssorhynchus) darlingi (539)	Anopheles (Nyssorhynchus) darlingi (106) nuneztovari (21) oswaldoi (19) (Anopheles) peryassu (2) mediopunctatus (19) shannoni (1) Aedes fulvus (2) Psorophora cingulata	Anopheles (Nyssorhynchus) darlingi (78) nuneztovari (4) oswaldoi (3) (Anopheles) peryassu (1) mediopunctatus (7) (Stethomyia) nimbus(1) Aedes fulvus (2) Culex spisspes (1) Psorophora cingulata (3)

TABLE 24

Number of ♀ ♀ Anopheles darlingi Root collected in 5 min. resting captures inside and outside of an experimental house at Floresta, Ituxi River, Amazonas, Brazil. Collections were conducted by 1 collector each 28 February - 1 March 1975.

Time	Number found resting by site		
	Inside house wall	Outside house wall	Vegetation
1830-1835[a]	0	0	0/1
1830-1835[b]	0	1/0	0/1
1900-1905[a]	0	0	0/1
1900-1905[b]	0/3	1/0	0/1
1930-1935[a]	0	0/3	0/2
1930-1935[b]	0/1	0/7	0/5
2000-2005[a]	1/0	0	0/12
2000-2005[b]	1/0	0/2	0/2
2030-2035[a]	2/0	0/4	0/1
2030-2035[b]	1/0	0/3	2/6
2100-2105[a]	1/0	0	0/2
	6/4	2/19	2/34
0540-0545[b]	1/0	0/1	2/0
0610-0615[b]	0	0	1/0
0640-0645[b]	0	0	1
0710-0715[b]	1/0	0	0
	2/0	0/1	3/0

[a] Collections conducted 28 February 1979.

[b] Collections conducted 1 March 1979.

exit trap data (n = 53, a = 163 and r = 15.5). The similarity between these population estimators is interesting but does not prove degree of accuracy. Additional studies are required to fully understand the variables involved with this study method.

Collections to study the temporal and spatial distribution of An. darlingi away from the house during the early evening activity interval were conducted during study number 2. Collections were conducted for 15 min, each at different distances from the house, viz., < 10, 20 and 40 meters from the house. Prior to sunset the greatest number were collected furtherest from the house, but all subsequent collections were uniformly high near the house compared to the more remote collecting sites (Table 22). The crepuscular peak in activity was clearly revealed near the house, but not at 20 m and 40 m from the house. Only An. darlingi were collected near the house, whereas we observed a considerable increase in species diversity and a great decrease in numbers of darlingi at 20 m and 40 m from the house (Table 23).

Collections of resting darlingi were conducted inside and outside the house from 1830 - 2105 and 0540 - 0715. The females collected inside the house prior to 1935 hr were unfed, but all subsequent specimens were engorged (Table 24). The majority of specimens caught outside were unfed during the evening. However, three of 4 specimens collected resting outside in the morning were engorged.

c. The Role of Euglossine Bees in the Removal of DDT from Sprayed Houses.

1) Objectives: To determine the impact of prolonged bee activity on the residue levels of DDT on walls of sprayed houses.

2) Background:

The strong insecticidal activity of DDT (dichlorodiphenyltrichloroethane) was first demonstrated in 1943 (24). DDT was subsequently employed throughout the world as a "front line defense" against insects of agricultural or public health importance, and is still widely used for residual treatment of house walls for control of mosquitoes in Brazil.

While studying the ecology of the malaria vector Anopheles (Nyssorhynchus) darlingi Root along the Ituxi River, Amazonas, Brazil, we observed aggreagates of male bees on the walls of houses that are routinely treated with DDT. The possibility of DDT being an insect attractant is incongruous with its premier role as an insecticide. Thus, our curiosity was aroused when we discovered that these Euglossine bees, identified as Euplusia purpurata, were

well known to the residents as the insects that eat DDT ("o bicho que come DDT").

The euglossine bees have been the subject of many fascinating studies due to their important role as plant pollinators. Both males and females provide valuable pollination services in their pursuit of nectar. An even more specific relationship has been found for the males in their apparent pursuit of certain flower odors, i. e., many flowers attract only one species of bee that affects pollination (26). The flower visiting behavior of the males has been described as follows:

> "When they visit an euglossine flower, they brush on the surface of the flower with pads of hair on the forefeet. The bees characteristically brush for a short time and they hover near the flower while scrubbing their legs together and evidently placing some substance in their inflated hind tibiae. The bees usually repeat this behavior several or many times, sometimes remaining as long as 90 minutes at one inflorescence" (26).

3) Progress:

We collected several bees while they were busily brushing their foretarsi on the DDT-treated walls of houses. Thirty or more constantly active bees would be seen at one time, and as some would leave, others would enter. The occasional presence of very shallow grooves at the site of bee activity indicated that perhaps the heavily sclerotized mandibles were sometimes employed to collect the DDT residues. The residents stated that these bees appear after houses are sprayed with DDT and are most abundant immediately after treatment.

Five of the bees we collected were sent to the U.S. Army Environmental Hygiene Agency, Aberdeen Proving Ground, Md., where they were dissected and each body part separately analyzed for DDT residues in accordance with analytical procedures recomended by the USEPA (27). The head, thorax, abdomen and fore-, mid- and hindlegs were processed for each specimen (wings were discarded). The body parts were weighed, prior to DDT extraction, so concentrations of insecticide could be expressed in parts per million (ppm) by body weight.

Total residue in µg/bee for 5 DDT isomers are presented in Table 25. Clearly the p,p' - DDT isomer was present consistently in the greatest concentrations. The sum of the DDT isomers expressed

TABLE 25

DDT residues from 5 males of Euplusia purpurata collected from sprayed houses along the Ituxi, River, Amazonas, Brasil.

DDT	Residue concentrations (ub/bee) for 5 DDT isomers				
	Bee No.1	Bee No.2	Bee No.3	Bee No.4	Bee No.5
o,p' - DDD	2.32	4.59	0.80	*	*
p,p' - DDD	24.99	69.47	44.01	32.49	80.45
p,p' - DDE	16.02	52.24	36.38	28.64	58.84
p,p' - DDT	135.25	472.53	174.70	11.21	335.95
p,p' - DDT	985.80	2944.72	2087.35	1545.50	2630.54
DDTR	1169.32	3557.95	2352.50	>1654.81	>3122.06

*
Part of the extract lost during centrifugation in the laboratory.

as DDTR*, converted to parts per million by dry weight, revealed exceptionally high concentration of DDT in these Brazilian bees (Table 26). Highest concentrations were consistently found in the hindlegs, with smaller amounts in the fore- and midlegs. Although significant residue levels were found in head, thorax and abdomen, DDT concentrations in these regions were markedly less than what was found in the hind-legs. For honey bees, the average LD_{50} (lethal dose for 50% of test populations) for p,p' - DDT alone is 5.6 ug/bee (28). Although it is inaccurate to compare values from one species to another, these statistics provide a perspective for evaluating the high residue levels found in the Brazilian bees. Looking at total p,p' - DDT in µg/bee, for instance, there was a low of 985 in bee 1 and a high of 2944.72 in bee 2, or 176.0 to 525.8 times greater than the LD_{50} value for honey bees. Since no dead or moribund bees were seen at the collections sites, the high residue levels of DDT suggest a high degree of resistance in these euglossine bees. Bees included in this analysis were collected at 3 isolated houses separated by 1-2 days travel by boat and it seems certain that the DDTR residue found in the bees was a direct result of contact with the sprayed house walls.

We have considered the possibility that males of E. purpurata enter houses because of attractants in the house construction materials or in response to carriers in the DDT formulation. For the latter, it is relevant to note that attractants to male euglossine bees are characteristically aromatics (28, 29). The DDT sprayed in houses along the Ituxi River is applied as a wettable powder formulation, composed of DDT, talc and water. Clearly, DDT is only aromatic in this mixture. In addition, males of E. purpurata have been observed scratching on boards treated with another aromatic insecticide, Aldrin (29). Regarding the possibility of attractants in house construction materials, houses along the river are constructed mainly of local forest products, viz., deciduous trees for the frame, palm thatching for the roof and palm slats for the walls and floors. Although the bees seen to prefer the ceiling, they frequently observed brushing on all types of house construction materials. In one case, they were found brushing on a tree trunk, in the forest, that had been spot-sprayed with DDT.

Bees have been reported to enter houses after DDT applications by residents near Manaus, Amazonas, Brazil. They also have been

---

* DDTR is a means of summing all the DDT isomers and expressing the total in terms of DDT. The following equation is utilized: [(o,p' + p,p' - DDD) + (0,p' _ p,p' - DDE)] + 1.114 + (o,p' + p,p' - DDT).

TABLE 26

Residue levels of DDTR in male Euplusia purpurata collected from sprayed houses along the Ituxi, River, Amazonas, Brazil.

Bee No.	Residue levels of DDTR in ppm or ub/g						
	Forelegs	Midlegs	Hindlegs	Head	Thorax	Abdomen	Total ppm ug/g/bee
Bee No. 1	451.87	930.14	10,526.15	123.58	29.95	118.78	12,180.48
Bee No. 2	1,266.54	4,245.42	29,082.55	369.02	60.47	203.15	35,227.14
Bee No. 3	2,464.81	6,222.27	11,203.44	839.04	153.87	121.08	21,004.
Bee No. 4	2,768.94	1,685.38	10,631.66	———	99.11	———	15,185
Bee No. 5	10,997.78	3,706.23	25,025.52	1,326.07	399.26	172.65	41,627.52

observed brushing on house walls near Iquitos, Columbia (personal communication, Dr. R. L. Dressler, Smithsonian Tropical Research Institute, Box 2072, Balboa, Canal Zone). That this is a widespread phenomenon was further verified by a recent report on the sightings of E. purpurata within treated houses in many areas in the state of Para, Brazil (30). However, no documentation of these insects actually collecting insecticide has been made and this is the first such report.

Based on the distribution of DDT residues in the bees, it seems that they do not seek the insecticide for strictly dietary purposes. Thus, we suspect that E. purpurata males are attracted by the odor of DDT which then stimulates their behavior of brushing the insecticide from walls for storage in a pouch of the hind tibia (saccate hind tibial organ).

d. Comments:

In summary, we have documented exophilic and endophagic behavior patterns for An. darlingi populations at the Ituxi River study area. Even in the unsprayed experimental house at Floresta the An. darlingi do not rest inside the house for more than a few hours. Additional observations are as follows:

1) Anopheles darlingi are consistently present at most of the single and multiple family habitations along the Ituxi River systems;

2) The An. darlingi populations demonstrate peak host-seeking activity at sunset and sunrise in the peridomiciliary environment;

3) Activity, in the absence of perturbations from low ambient temperatures, within a house with complete walls is relatively constant throughout the night;

4) Biting activity in a partially enclosed house (with one wall) reflects the bimodal pattern documented for the peridomiciliary environment;

5) The spatial distribution of host-seeking darlingi populations is clumped near to and within the experimental house;

6) The preferred, within house, resting site of engorged An. darlingi is on the ceiling.

The latter observations is in sharp contrast to the finding of Deane and Damasceno that 87.2% of the An. darlingi collected were resting on the lower 2 m of the house walls. Since walls are frequently not over 2 m in height, with consequent large openings between the roof

and walls, selection of the ceiling resting sites may facilitate escape from the house. Also it is interesting that E. purpurata seem to preferentially collect DDT (see section c) from the ceiling and upper levels of the wall.

The objective for future studies is to determine the influence of DDT treatment on the behavioral parameters described in this report. In some cases we will seek further verification of our observations prior to spraying the experimental house with DDT. However, a control house, that will not be sprayed, is under construction. It is important to identify the main points of entry and exit of An. darlingi from the house and to elucidate the variability in activity as a result of different portals of exit. More emphasis will be placed on studies of resting sites and host preferences. These efforts will be facilitated by the employ of a recently constructed vacuum aspirator. More detailed studies will be conducted to determine a) the impact of the euglossine bees in DDT removal and b) characterize, in more detail, their insecticidophilic behavior.

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